

**UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CÂMPUS DE JABOTICABAL**

**DINÂMICA DE SERAPILHEIRA E VOLATILIZAÇÃO DE NH_3
EM PASTOS DE CAPIM-MARANDU COM E SEM
NITROGÊNIO OU CONSORCIADOS COM AMENDOIM
FORRAGEIRO**

Vanessa Zironi Longhini
Zootecnista

2020

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FORRAGEIRO**

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

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TÍTULO DA TESE: DINÂMICA DE SERAPILHEIRA E VOLATILIZAÇÃO DE NH_3 EM PASTOS DE CAPIM-MARANDU COM E SEM NITROGÊNIO OU CONSORCIADOS COM AMENDOIM FORRAGEIRO

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Jaboticabal, 30 de março de 2020

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Epígrafe

“Thus, the task is not so much to see what no one yet has seen, but to think what nobody yet has thought about that which everybody sees”.

Arthur Schopenhauer

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Aos meus amados pais Sidemar e Mirtes, e à minha irmã Vivian, por todo amor, exemplo de vida, incentivo, conselhos e orações. Ao meu noivo Luan pela paciência, amor e auxílio durante essa jornada. A vocês minha mais sincera e eterna gratidão!

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“Every person that comes into our life comes for a reason; some come to learn, and others come to teach”.

Antoine de Saint-Exupéry

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CERTIFICADO

Certificamos que o Protocolo nº 10356/14 do trabalho de pesquisa intitulado **"Pastagem de capim-marandu manejada com fertilizante nitrogenado ou em consórcio com leguminosa na mitigação de gases de efeito estufa"**, sob a responsabilidade da Prof.^a Dr.^a Ana Cláudia Ruggieri está de acordo com os Princípios Éticos na Experimentação Animal adotado pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em reunião ordinária de 07 de julho de 2014.

Jaboticabal, 07 de julho de 2014.

Prof.^a Dr.^a Paola Castro Moraes
Coordenadora – CEUA

DINÂMICA DE SERAPILHEIRA E VOLATILIZAÇÃO DE NH_3 EM PASTOS DE CAPIM-MARANDU COM E SEM NITROGÊNIO OU CONSORCIADOS COM AMENDOIM FORRAGEIRO

RESUMO – A inclusão de leguminosas em sistemas de gramíneas têm sido utilizadas com o objetivo de reduzir ou substituir a adubação nitrogenada, por meio da fixação biológica de nitrogênio atmosférico, via simbiose entre leguminosas-bactérias. Além disso, melhoras no aproveitamento do alimento pelo animal podem ocorrer, diminuindo a quantidade de nitrogênio excretado ao ambiente, pela urina e fezes. A volatilização de amônia nas excretas bovinas provenientes de pastos tropicais é pouco conhecida, e menos ainda sobre pastos tropicais em consórcio com leguminosa. Diante do exposto, esta tese teve como objetivo avaliar o uso do amendoim forrageiro (*Arachis pintoï* Krapov. & W.C. Greg) em consórcio com capim-marandu [*Urochloa brizantha* (Hochst. Ex A. Rich.) R. D. Webster cv. 'Marandu'] como estratégia de substituição ao uso do adubo nitrogenado ureia em sistemas de produção de forragem. Para isso, três estudos foram realizados. O primeiro estudo avaliou respostas da forragem à diferentes entradas de N e tipos de forragem durante o estabelecimento sob lotação rotacionada. Os tratamentos incluíram o amendoim forrageiro em consórcio com capim-marandu, capim-marandu sem adubação nitrogenada e capim-marandu adubado com ureia ($150 \text{ kg N ha}^{-1} \text{ ano}^{-1}$). O segundo estudo avaliou como as estratégias de manejo de pastos de capim-marandu afetam as taxas de deposição e decomposição da serapilheira, bem como a composição química da serapilheira dois anos após a semeadura. Os tratamentos foram os mesmos avaliados no primeiro estudo. No terceiro estudo, realizamos três experimentos de campo (estação chuvosa, de transição e seca) para quantificar as emissões de amônia (NH_3), simulando a aplicação de urina e fezes de novilhas, e ureia em pastos de capim-marandu no Brasil. As excretas das novilhas foram provenientes dos tratamentos do primeiro estudo, ou seja, pastos de capim-marandu sob três formas de suprimento de N (0, 150 kg N ha^{-1} ou em consórcio com leguminosa). O adubo nitrogenado aumentou a massa foliar do capim-marandu ($2470 \text{ kg MS ha}^{-1}$) em comparação aos tratamentos não adubado e consorciado com amendoim forrageiro (1700 e $1310 \text{ kg MS ha}^{-1}$, respectivamente). A porcentagem de

N derivado da atmosfera (% Ndfa) para o amendoim forrageiro variou de 79 a 85% e a fixação biológica de N₂ (BNF) variou de 0,7 a 2,6 kg N ha⁻¹ ciclo⁻¹. A adubação nitrogenada aumentou a massa existente e a taxa de deposição de serapilheira (2380 e 21 kg ha⁻¹ MO, respectivamente) em comparação com o capim-marandu em monocultura (1670 e 16 kg ha⁻¹ MO, respectivamente) ou em consórcio com o amendoim forrageiro (1470 e 17 kg ha⁻¹ MO, respectivamente); ambas as variáveis foram afetadas pelas condições climáticas, com baixa taxa de deposição da serapilheira e massa existente nos meses com pouca chuva (GC4). O capim-marandu adubado com N apresentou maior concentração de N na serapilheira ($7,9 \pm 0,4$ g kg⁻¹ MO), resultando em menor relação C:N ($34,0 \pm 2,8$) do que em pastos de capim-marandu em monocultura ou consorciados com a leguminosa ($44,1$ e $57,7 \pm 2,8$, respectivamente). As perdas de N aplicado na forma de NH₃ entre as estações variaram entre 7,6 e 16,6% (média 11,7%) na urina, entre 1,4 a 2,9% (média 2,0%) nas fezes, e entre 11,2 a 20,5% (média 14,8%) na ureia, mostrando que a volatilização de NH₃ na urina foi significativamente maior do que nas fezes, em todas as estações. A emissão de NH₃ nas excretas de novilhas sob pastejo em pastos de capim-marandu consorciado com amendoim forrageiro apresentaram menor emissão de NH₃ do que nas excretas provenientes de novilhas mantidas em pastos de capim-marandu adubado com nitrogênio. No geral, os benefícios do amendoim forrageiro foram mínimos porque a porcentagem de leguminosas foi ~10%. A adubação nitrogenada aumentou a taxa de deposição da serapilheira, bem como a massa existente e a concentração de N, enquanto a baixa proporção da leguminosas no sistema consorciado não foi suficiente para melhorar a ciclagem de nutrientes no ecossistema de pastagem. Excretas das novilhas e ureia depositadas em pastos de clima tropical apresentaram maiores emissões de NH₃ principalmente na estação chuvosa.

Palavras-chave: amendoim forrageiro, ciclagem de nutrientes, emissão de amônia, manejo de pasto, ureia.

LITTER DYNAMICS AND NH₃ VOLATILIZATION IN MARANDU GRASS PASTURE WITH AND WITHOUT NITROGEN OR MIXED WITH FORAGE PEANUT

ABSTRACT – Legume inclusion in grass systems may reduce or replace the nitrogen fertilization, through the biological fixation of atmospheric nitrogen by the legume-bacterial symbiosis. Furthermore, improvement in animal nutrition can occur, decreasing the amount of nitrogen excreted into the environment via urine and dung. There have been few studies to quantify ammonia emissions from bovine excreta deposited in tropical pastures, and even fewer in warm-season grass-legume mixtures. Therefore, this thesis aimed to evaluate the use of forage peanut (*Arachis pinto* Krapov. & W.C. Greg) mixed with palisadegrass [*Urochloa brizantha* (Hochst. ex A. Rich.) R. D. Webster cv. ‘Marandu’] as a strategy to replace nitrogen fertilizer in forage production systems. Three studies were performed. The first study evaluated herbage responses to N inputs and forage types during establishment under rotational stocking. Treatments included palisadegrass-forage peanut mixture, unfertilized palisadegrass, and palisadegrass fertilized with urea (150 kg N ha⁻¹ yr⁻¹). The second study evaluated how sward management strategies of palisadegrass pastures affected the litter deposition and decomposition rates, as well as litter chemical composition two years after seeding. The treatments were the same as those evaluated in the first study. In the third study, we conducted three field trials (wet, transition, and dry season) to quantify the ammonia (NH₃) emissions, simulating application of heifer urine, dung, and urea fertilizer on warm-climate grasslands in Brazil. Heifer excreta were derived from the treatments of the first study, i.e. pastures of palisadegrass under three forms of N supply (0 or 150 kg N ha⁻¹ or mixed with legume). Nitrogen fertilizer increased leaf mass (2470 kg DM ha⁻¹) compared with the control and mixed treatments (1700 and 1310 kg DM ha⁻¹, respectively). The percentage of N derived from atmosphere (%Ndfa) for forage peanut ranged from 79 to 85% and the biological N₂ fixation (BNF) input ranged from 0.7 to 2.6 kg N ha⁻¹ cycle⁻¹. Nitrogen fertilization increased existing litter mass and litter deposition rate (2380 and 21 kg ha⁻¹ OM, respectively) compared with grass in monoculture (1670 and 16 kg ha⁻¹ OM, respectively) or legume-grass mixtures (1470 and 17 kg ha⁻¹ OM, respectively); both variables were affected by weather conditions, with low litter

deposition rate and low existing litter in the grazing cycles with poor rainfall (GC4). Nitrogen fertilized palisadegrass had greater litter N concentration ($7.9 \pm 0.4 \text{ g kg}^{-1}$ OM) resulting in lower C:N ratio (34.0 ± 2.8) than in monoculture or legume-grass mixtures (44.1 and 57.7 ± 2.8 , respectively). Nitrogen applied lost as NH_3 emission across seasons ranged between 7.6 to 16.6% (mean 11.7%) for urine, 1.4 to 2.9% (mean 2.0%) for dung, and 11.2 to 20.5% (mean 14.8%) for urea, showing that NH_3 emission from urine was significantly greater than from dung, for all seasons. Heifer excreta from grass-legume mixture had lower NH_3 emission than excreta from the N fertilized pasture. Urea fertilizer broadcast on palisadegrass presented greater NH_3 emissions in the wet season compared with the dry season but did not differ from the transition season. Overall, benefits from the forage peanut were minimal as legume percentage was $\sim 10\%$. Nitrogen fertilization increased litter deposition rate as well existing litter mass and litter N concentration, whereas the low proportion of legume in the grass-legume mixtures was not enough to enhance litter nutrient cycling in the grassland ecosystem. Heifer excreta and urea fertilizer deposited on warm-climate grasslands increased the NH_3 emission mainly in the wet season.

Keywords: ammonia emission, nutrient cycling, pasture management, forage peanut, urea.

LISTA DE ABREVIATURAS

- %Ndfa** Percentage of nitrogen derived from the atmosphere/ Porcentagem de nitrogênio derivado da atmosfera
- BNF/ FBN** Biological dinitrogen fixation/ Fixação biológica de nitrogênio
- C** Carbon/ Carbono
- C:N ratio** Carbon:Nitrogen ratio/ relação Carbono:Nitrogênio
- Ca(NO₃)₂** Calcium nitrate/ Nitrato de cálcio
- CO(NH₂)₂** Urea/ Ureia
- CO₂** Carbon dioxide/ Dióxido de carbono
- CP/ PB** Crude protein/ Proteína bruta
- D** Day/ Dia
- DAA** Days after application of treatments/ Dias após aplicação dos tratamentos
- DM** Dry matter/ Matéria seca
- GC** Grazing cycle/ Ciclo de pastejo
- GHG/ GEE** Greenhouse gases/ Gases de efeito estufa
- H⁺** Hydrogen ion/ Íon de hidrogênio
- IPCC** Intergovernmental Panel on Climate Change/ Painel Intergovernamental de Mudanças Climáticas
- KNO₃** Potassium nitrate/ Nitrato de potássio
- L:S** Leaf:stem ratio/ relação folha:colmo
- LD_{14d}** Litter deposited after 14 d/ serapilheira depositada após 14 d
- LI** pre-grazing canopy light interception/ Interceptação luminosa pré pastejo
- N** Nitrogen/Nitrogênio
- N₂** Nitrogen atmospheric/ Nitrogênio atmosférico
- N₂O** Nitrous oxide/ Óxido nitroso
- NH₃** Ammonia/ Amônia
- NH₄⁺** Ammonium/ Amônio
- NH₄NO₃** Ammonium nitrate/ Nitrato de amônio
- (NH₄)₂CO₃** Ammonium carbonate/ Carbonato de amônio
- (NH₄)₂SO₄** Ammonium sulfate/ Sulfato de amônio
- SM** Sward management strategies/ Estratégias de manejo de pasto

TAGN Total aboveground nitrogen/ Nitrogênio total acima do solo

TAGN_{PG} Total aboveground nitrogen for palisadegrass/ Nitrogênio total acima do solo para o capim-marandu

TAGN_{FP} Total aboveground nitrogen for forage peanut/ Nitrogênio total acima do solo para o amendoim forrageiro

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CAPÍTULO 1 – Considerações gerais

1. INTRODUÇÃO

Dos tipos de vegetação existentes, as pastagens apresentam melhor distribuição territorial, cobrindo 20% da superfície terrestre global (ZHOU et al., 2020). No Brasil, 159 milhões de hectares são destinados a áreas de pastagem (IBGE, 2017), sendo o pasto, a base alimentar na produção bovina, considerado como a forma mais econômica para o desenvolvimento da pecuária brasileira (DIAS-FILHO, 2014).

As condições das pastagens brasileiras avaliadas pelo IBGE (2017) variam, podendo essas apresentarem algum grau de degradação pelo manejo inadequado ou pela falta de reposição nutricional, bem como pastagens em boas condições, incluindo aquelas em processo de recuperação. O grau de degradação pode ser afetado por fatores climáticos, como secas prolongadas, erosões eólicas ou pluviais, e por fatores ligados às práticas de manejo de pasto inapropriadas, como superpastejo, altas taxas de lotação, ataques de insetos ou pragas, ausência de reposição nutricional, escolha da forrageira, e a exigência nutricional da forrageira (DIAS-FILHO, 2014; ZHOU et al., 2020). Em sistemas consorciados, a escolha da leguminosa e da gramínea se tornam fundamentais para a persistência das forrageiras e para evitar os processos de degradação (BOURSCHEIDT et al., 2019).

O uso da adubação nitrogenada ou consórcio entre gramíneas e leguminosas são considerados como ferramentas no manejo do pasto, fornecendo melhorias na interação solo-planta-animal. O manejo do pasto visando o retorno dos nutrientes ao solo, torna-se importante para reduzir o declínio das pastagens, evitando o início dos processos de degradação (BODDEY et al., 2004).

A adubação nitrogenada tem como objetivo, aumentar a produtividade da forragem, melhorar o valor nutricional do pasto, e consequentemente aumentar o ganho de peso animal (DELEVATTI et al., 2019). Apesar dos diversos benefícios, o uso excessivo de adubos nitrogenados químicos, resultam em impactos ambientais, principalmente por serem oriundos da queima de combustíveis fósseis, contribuindo com a emissão de gases de efeito estufa (GEE) (HASLER et al., 2015) e volatilização

de amônia (NH₃) após aplicação nas pastagens (DUBEUX e SOLLENBERGER, 2020).

A inclusão da leguminosa nos sistemas de gramíneas é considerada como uma alternativa ao uso de adubos nitrogenados, com o intuito de reduzir os impactos ambientais, tornando os sistemas mais sustentáveis (MUIR et al., 2011; MUIR et al., 2017). Outro benefício que pode ocorrer ao sistema, é melhorar o retorno de nutrientes ao solo, via decomposição da serapilheira (KOHMANN et al., 2019; DUBEUX e SOLLENBERGER, 2020) e deposição de urina e fezes de animais nas pastagens (DUBEUX e SOLLENBERGER, 2020), sendo a composição química dessas excretas influenciadas pela dieta fornecida ao animal (HRISTOV et al., 2013). No entanto, excretas bovinas são consideradas como fontes primárias na volatilização da amônia (PHELAN et al., 2015). Embora a amônia não seja considerada como GEE, pode ser utilizada como fonte indireta na formação de óxido nitroso (N₂O) (IPCC, 2019), e deve ser quantificada em todos os sistemas de pastagem.

Portanto, o presente estudo abordará informações voltadas às características morfológicas de pastos de capim-marandu adubados com ureia ou em consórcio com amendoim forrageiro, bem como a formação da serapilheira, e a quantificação da volatilização da amônia nas excretas de novilhas mantidas nesses sistemas de pastagens.

2. REVISÃO DE LITERATURA

2.1 Manejo de pastos de capim-marandu

No Brasil, o gênero *Urochloa* (syn. *Brachiaria*) ocupa 80 milhões de hectares de pastagens (ZIMMER et al., 2012), sendo o capim-marandu (*Urochloa brizantha* cv. Marandu) o cultivar mais utilizado, cobrindo 56% desses 80 milhões de hectares (SILVA et al., 2016). A escolha por esse cultivar ocorre por inúmeras razões, como alta produção de matéria seca, variando entre 5 a 11 t ha⁻¹, proteína bruta entre 11 a 18% e digestibilidade da matéria seca entre 56 a 68% (BARBERO et al., 2015; DELEVATTI et al., 2019). Essas variações podem ocorrer de acordo com o tipo de manejo de pasto estabelecido, como altura de entrada adotada (BARBERO et al.,

2015), uso de adubação nitrogenada (DELEVATTI et al., 2019), taxa de lotação (REZENDE et al., 2008), consórcio com leguminosa (ALENCAR et al., 2018; GOMES et al., 2018), sistema silvipastoril (NASCIMENTO et al., 2019), uso de interceptação luminosa ou período de descanso para dar início ao pastejo (GOMES et al., 2018), entre outros.

O manejo do pasto juntamente com fatores edafoclimáticos afetam a quantidade e o valor nutricional da forragem (GOMIDE e GOMIDE, 2013). Essas ferramentas são utilizadas por produtores rurais, com o intuito de aumentar o desempenho animal, bem como em manter a longevidade e a produtividade da pastagem, evitando os processos de degradação (BODDEY et al., 2004).

Dentre os manejos acima citados, a adubação do pasto é uma prática de reposição dos nutrientes extraídos pelo consumo animal, que reflete no aumento da produção de forragem, melhora no valor nutricional da forragem e consequentemente no desempenho animal (DELEVATTI et al., 2019). O nitrogênio (N) fornecido pela adubação afeta no aumento da taxa de renovação dos tecidos das plantas e no alongamento foliar, resultando em maior acúmulo de forragem (FAGUNDES et al., 2006; PEREIRA et al., 2015). No entanto, o uso em excesso do N nas pastagens pode trazer prejuízos econômicos e ambientais.

A introdução da leguminosa em consórcio com gramíneas tem sido recomendado como uma das melhores alternativas para reduzir o uso de adubos nitrogenados, e assim alcançar sistemas de pastejo mais sustentáveis (MUIR et al., 2011; MUIR et al., 2017). Dentre as leguminosas utilizadas para pastejo, o amendoim forrageiro (*Arachis pintoi*) torna-se uma opção vantajosa para o consórcio com gramíneas tropicais. Embora apresente estabelecimento inicial lento (ARGEL e PIZARRO 1992), possui persistência em consórcios com forrageiras de hábito de crescimento rápido como a *Urochloa* (ANDRADE et al., 2012). Em virtude da inclusão da leguminosa no sistema, o estudo dos fluxos de N (contribuição da FBN, reciclagem do N via animal e via deposição da serapilheira) visando a obtenção de um balanço de N, se torna de suma importância dentro de sistemas consorciados.

2.2 Adubação nitrogenada

O nitrogênio é um dos nutrientes mais limitantes no crescimento e renovação de tecidos das plantas (BOURSCHEIDT et al., 2019), constitui os aminoácidos, proteínas, ácido nucleicos, nucleotídeos e coenzimas (TAIZ e ZEIGER, 2002). Atua em processos de absorção iônica, respiração, multiplicação, diferenciação celular e fotossíntese (CARMELLO, 1999). No entanto, o nitrogênio disponível no solo não é suficiente para suprir a demanda das forragens, sendo necessária a reposição desse nutriente (FAGUNDES et al., 2005). A introdução do nitrogênio ao sistema de pastagem pode ocorrer de diversas maneiras, como pela adubação nitrogenada, deposição atmosférica (raios ou chuvas), fixação biológica por simbiose entre bactéria-leguminosa, e decomposição da serapilheira ou excreta animal por meio da ciclagem de nutrientes (DUBEUX et al., 2013; BOURSCHEIDT et al., 2019).

De modo geral, a adubação nitrogenada em pastos tropicais é a forma mais prática e rápida de fornecer o nutriente ao sistema (BOURSCHEIDT et al., 2019), trazendo diversos benefícios ao pasto, como aumento na taxa de aparecimento foliar, taxa de alongamento foliar, índice de área foliar e a densidade de perfilhos, o que resulta em maiores taxas de acúmulo de forragem (FAGUNDES et al., 2006; PEREIRA et al., 2015; YASUOKA et al., 2017; SALES et al., 2019). Melhora nos aspectos nutricionais da planta também são observados, como aumento no teor de proteína bruta e digestibilidade (SOLLENBERGER et al., 2009; SILVA et al., 2016; DELEVATTI et al., 2019).

Aspectos relacionados à dose, fonte e parcelamento do adubo nitrogenado devem ser levados em consideração, para que a prática se torne viável e eficiente (COSTA et al., 2006). Nesse sentido, Delevatti et al. (2019) verificaram que pastos de capim-marandu adubados com doses superiores a 90 kg N ha⁻¹ resultam em menor produção de forragem por kg N aplicado. No mesmo estudo, a adubação de 180 kg N ha⁻¹ resultou em um incremento de 3 arrobas ha⁻¹ no ganho de peso de bovinos, em comparação com a dose de 90 kg N ha⁻¹, enquanto essa resultou no incremento de 9 arrobas ha⁻¹ em comparação a pastos não adubados. Dessa forma, dobrar a dose da adubação nitrogenada não foi uma prática vantajosa, resultando em menor custo benefício para o produtor.

De acordo com Sales et al. (2019), doses de adubação nitrogenado entre 50 a 75 kg N ha⁻¹ ciclo⁻¹ em pastos de capim-marandu resultam em maior produção e acúmulo de forragem, demonstrando que o parcelamento de doses maiores devem ser realizadas para minimizar as perdas do nitrogênio para o ambiente. Dupas et al. (2016) comparando diferentes fontes de adubos nitrogenados em pastos de capim-marandu não verificaram diferenças na produção total de forragem, no entanto, indicam o uso de adubos com menores perdas de nitrogênio para o ambiente, como sulfonitrato de amônio, ureia com inibidor de urease e ureia revestida com polímero.

Dentre os adubos nitrogenados, a ureia apresenta menor custo por quilograma de nitrogênio (BOURSCHEIDT et al., 2019), sendo a fonte mais utilizada, com o consumo de aproximadamente 5 milhões de tonelada no ano de 2017 (ANDA, 2017). De acordo com a Scot Consultoria, no Estado de São Paulo, a tonelada da ureia em janeiro de 2020 foi cotada a R\$ 1.562,24 (sem contabilizar o frete), esse valor pode aumentar de acordo com o tamanho e a localização da propriedade do produtor.

A produção de adubos nitrogenados no Brasil não é suficiente, sendo necessário importações do produto para suprir a demanda do país (MME, 2019). A produção desses adubos tem como matéria-prima a amônia, procedente da reação do N do ar atmosférico com o hidrogênio proveniente de diversas fontes, como gás natural, derivados do petróleo ou óleo combustível (MME, 2019). Para produzir 1 tonelada de ureia, utiliza-se em média 6 barris de petróleo (HUNGRIA et al., 2007). Dessa forma, por serem provenientes da queima de combustível fóssil, contribuem para a emissão de GEE, tanto no momento de sua fabricação como durante o transporte e aplicação nas pastagens e lavouras. O uso excessivo pode promover, a contaminação do lençol freático pela lixiviação de nitrato, a volatilização de NH₃ e a emissão de N₂O (ZAMAN et al., 2009).

Como estratégia às fontes químicas, a utilização de consórcios entre leguminosas e gramíneas apresentam vantagens na melhoria do valor nutricional das pastagens (OLIVEIRA et al., 2010), principalmente na época da seca, por elevar o teor de proteína bruta do volumoso na alimentação dos animais em pastejo (EVANGELISTA et al., 2003).

2.3 Amendoim forrageiro (*Arachis pintoï*)

O gênero *Arachis* é originalmente encontrado na América do Sul, com a existência de aproximadamente 80 espécies, sendo 68 dessas encontradas no Brasil (FERREIRA et al., 2012). No Brasil, as espécies registradas no Ministério da Agricultura, Pecuária e Abastecimento são *Arachis pintoï* Krapov. & W. C. Greg. e *Arachis repens* Handro (MAPA, 2017). *Arachis pintoï* é considerada a espécie mais utilizada em pesquisa no Brasil (VALENTIM et al., 2003; HOMEM et al., 2017; ALENCAR et al. 2018), sendo a única com registro formal para uso em pastagens. A espécie *A. repens* é mais utilizada para paisagismo (MIRANDA et al., 2003), enquanto o *A. glabrata* não possui Registro Nacional de Cultivares no MAPA, sendo mais comumente utilizada no estado da Flórida – EUA (DUBEUX et al., 2017; JARAMILLO et al., 2018; SANTOS et al., 2018).

Conhecido popularmente como amendoim forrageiro, o gênero *Arachis* é considerado como uma leguminosa herbácea perene, apresenta porte baixo 20 a 60 cm de altura (Figura 1A), com crescimento estolonífero prostrado e lançamento de estolões horizontalmente (Figura 1B) (MIRANDA et al., 2008).



Figura 1. *Arachis pintoï* cv. 'Amarillo' (A) porte baixo e (B) crescimento estolonífero prostrado com lançamento de estolões horizontais. (Foto: Vanessa Z. Longhini).

Inúmeras são as vantagens na utilização do amendoim forrageiro, como melhora na ciclagem de nutrientes, principalmente para sistemas consorciados com gramíneas, promovem a fixação do nitrogênio atmosférico (MIRANDA et al., 2008), pontos de crescimentos nos estolões são protegidos durante o pastejo animal

(ANDRADE et al., 2005), tolera sombreamento em até 50% da radiação fotossinteticamente ativa (GOBBI et al., 2009), produção de matéria seca varia entre 2 a 13 t ha⁻¹, 20% de proteína bruta, e 70% de digestibilidade (VALENTIM et al. 2003; CARVALHO et al., 2019; LIMA e ASSIS, 2019).

A fixação de N₂ atmosférico ocorre por meio da simbiose entre planta-bactéria, a qual está alojada em nódulos radiculares (SPRENT, 2007). Essa simbiose ocorre geralmente por bactérias do gênero *Bradyrhizobium* (MIRANDA et al., 2003; SÁ et al., 2019), as quais convertem o N atmosférico em amônia (JENSEN et al., 2012). Estudos desenvolvidos para cultivares de *A. pinto* em monocultura no Brasil, verificaram FBN variando entre 26 a 99 kg N ha⁻¹ ano⁻¹ (MIRANDA et al., 2003). Carvalho et al. (2019) encontrou valores superiores para o cultivar Belmonte, variando entre 88 a 244 kg N ha⁻¹ com o aumento da altura do dossel de 5 para 20 cm. Em estudos realizado com *A. glabrata* na Flórida, a FBN variou entre 123 a 280 kg N ha⁻¹ ano⁻¹ (DUBEUX et al., 2017). Em condições ambientais semelhantes ao estudo anterior, valores inferiores de FBN no *A. pinto* cv. 'Amarillo' em consórcio com *Paspalum notatum* cv. 'Pensacola' variaram entre 0 a 2,7 kg N ha⁻¹ colheita⁻¹ (JARAMILLO et al., 2018).

A limitação no uso das leguminosas na pecuária brasileira são influenciadas pela baixa oferta de sementes, bem como o elevado preço no mercado, as formas de propagação vegetativa dificultam a implantação de alguns cultivares, além do desconhecimento dos benefícios da leguminosa pelo produtor (LIMA e ASSIS, 2019). Por exemplo, os cultivares como Belmonte e Alqueire-1 são propagadas por meio de mudas, o que dificulta o processo de implantação, principalmente para grandes produtores. O cultivar Amarillo pode ser estabelecido por meio de sementes, no entanto, o alto custo das sementes e o estabelecimento inicial lento dificulta a escolha desse cultivar.

Atualmente, no estado de São Paulo, o preço de 1 kg de semente de *A. pinto* cv. 'Amarillo' é de R\$ 249,00 (BRSEEDS, 2019). Para o plantio de 1 hectare em sistema consorciado, são necessários 10 kg de sementes do amendoim forrageiro, resultando em um investimento de R\$ 2.490,00 + custos de implantação. Recentemente, a Embrapa-Acre lançou o cultivar BRS Mandobi (propagação por sementes), com a promessa de menores preços, tornando a utilização de

leguminosas uma prática mais acessível ao produtor rural (EMBRAPA, 2019). Resultados imediatos são difíceis de ocorrer, principalmente pelo lento estabelecimento do amendoim forrageiro comparado com gramíneas (TAMELE et al., 2018). No entanto, após o estabelecimento completo da leguminosa, preocupações como adubação nitrogenadas são minimizadas ou até mesmo inexistentes, tornando o sistema de pastagens mais sustentável.

2.4 Consórcio entre pastos de capim-marandu e amendoim forrageiro

A inclusão de leguminosas em sistemas de gramíneas tem como objetivo adicionar o nitrogênio atmosférico, por meio da fixação biológica, bem como melhorar o valor nutricional da dieta animal, favorecendo assim, o ecossistema solo-planta-animal (OLIVEIRA et al., 2010; DUBEUX et al., 2013; GOMES et al., 2018). Além disso, benefícios financeiros também ocorrem para o produtor rural, pela redução no uso de adubos nitrogenado (DUBEUX e SOLLENBERGER, et al., 2020). No entanto, para que a prática se torne eficiente e economicamente viável, a leguminosa escolhida deve apresentar persistência em consórcio com gramíneas (PEREIRA et al., 2020).

O amendoim forrageiro apresenta grande potencial para a utilização em consórcio com gramíneas tropicais (TAMELE et al., 2018), podendo ser consorciado com diversas gêneros, como *Urochloa* (ALENCAR et al. 2018; GOMES et al., 2018; TAMELE et al., 2018), *Cynodon* (RIBEIRO et al., 2008), *Penissetum* (CRESTANI et al., 2013) e *Panicum* (ANDRADE et al., 2006). Estudo realizado por Pereira et al. (2020) relataram aumento em 16% na taxa de lotação e ganhos de 30% no peso vivo animal ha⁻¹, para bovinos mantidos em pastos de capim-marandu consorciados com amendoim forrageiro, em comparação aos pastos adubados com 120 kg N ha⁻¹. Os mesmos autores verificaram ainda aumento na produção de forragem em 17% para os sistemas consorciados.

Em sistemas consorciados, o tipo de manejo a ser escolhido torna a atividade um pouco mais complexa, principalmente por existir duas espécies forrageiras com diferentes características anatômicas, fisiológicas e morfológicas crescendo sobre o mesmo ambiente (LEMPP, 2013). Nesse sentido, competições entre espécies

forrageiras por luminosidade, água e nutrientes do solo pode ocorrer (RUGGIERI et al., 2013). Segundo Tamele et al. (2018), a altura de desfolha do dossel forrageiro a 20 cm é ideal para garantir o desenvolvimento do amendoim forrageiro em consórcio com capim-marandu. De acordo com o mesmos autores, alturas superiores a 30 cm resultaram no aumento do estolão e pecíolo da leguminosa em busca de luz. Em sistemas rotativos, Gomes et al. (2018) verificaram que a interceptação luminosa (IL) como ferramenta de manejo em sistemas consorciados afeta o valor nutricional da forragem ingerida por bovinos. Os autores recomendam que o período de descanso deve ser interrompido em 90% IL e no máximo 95% de IL para garantir valores de proteína bruta 19.2% da matéria seca, 38% de fibra em detergente neutro e 69% digestibilidade para o amendoim forrageiro e para o capim-marandu 11% de proteína bruta, 58% de fibra em detergente neutro e 54% digestibilidade.

Estudos demonstraram que o amendoim forrageiro utiliza em torno 80% do nitrogênio atmosférico fixado para suprir suas demandas nutricionais de nitrogênio (DUBEUX et al., 2017; ALENCAR et al., 2018; SANTOS et al., 2018). Dessa maneira, a FBN em leguminosas desempenha papel fundamental na redução do uso de adubos nitrogenados. No entanto, para que os sistemas consorciados se tornem eficientes na quantidade de nitrogênio fixado, no mínimo 30% de leguminosa deve existir na composição botânica (LÜSCHER et al., 2014). Em estudo avaliando o estabelecimento do amendoim forrageiro sobressemeado em pastos de capim-marandu estabelecido, Alencar et al. (2018) encontraram 14% de amendoim forrageiro na massa de forragem, o que resultou em apenas 3,4 kg N ha⁻¹ mês⁻¹ de FBN. Nesse sentido, baixas proporções de leguminosas resultam em baixas quantidade de nitrogênio fixado ao sistema consorciado.

2.5 Deposição e decomposição da Serapilheira

O termo serapilheira pode ser definido como a camada de material vegetal morto presente na superfície do solo ou material vegetal morto presente no solo que não esteja aderido à planta viva (REZENDE et al., 1999; KRISHNA e MOHAN, 2017). Em áreas de pastagens com pouco ou nenhum tipo de reposição nutricional, práticas de manejo de pastagens, que favoreçam a qualidade e a quantidade de serapilheira

depositada, se tornam fundamentais para o retorno de nutrientes ao solo-planta (KRISHNA e MOHAN, 2017).

A produção de serapilheira e excreção animal são formas de retorno de nutriente ao solo, sendo a serapilheira considerada como uma das práticas mais eficientes, geralmente maior do que o retorno via deposição de urina e fezes animal (THOMAS, 1992). Isso porque, a distribuição da serapilheira ocorre de forma mais homogênea, enquanto as excretas, são depositadas de forma mais concentradas, em áreas utilizadas para o descanso animal, sombras, próximos de cochos e bebedouros (BODDEY et al., 2004; DUBEUX et al., 2006a), o que torna o retorno de nutrientes em áreas úteis da pastagem pequeno.

O retorno de nutrientes ao solo é dependente da quantidade de serapilheira depositada, da sua composição química e da taxa de decomposição (DUBEUX e SOLLENBERGER, 2020). Os processos de decomposição são afetados pela qualidade da serapilheira (ZHANG et al., 2008), a qual pode ser definida como a relação entre a composição química da serapilheira e a natureza dos compostos químicos (DUBEUX e SOLLENBERGER, 2020). A qualidade pode ser determinada pelos seguintes indicadores, C total, N total, P, N insolúvel em detergente ácido (NIDA), lignina, polifenóis e suas relações (THOMAS e ASAKAWA, 1993; DUBEUX e SOLLENBERGER, 2020). Sendo, serapilheiras de “alta qualidade” rapidamente decompostas enquanto as de “baixa qualidade” apresentam maior dificuldade de decomposição (COTRUFO et al., 2013). Uma representação gráfica do sistema de decomposição da serapilheira está ilustrada na Figura 2.

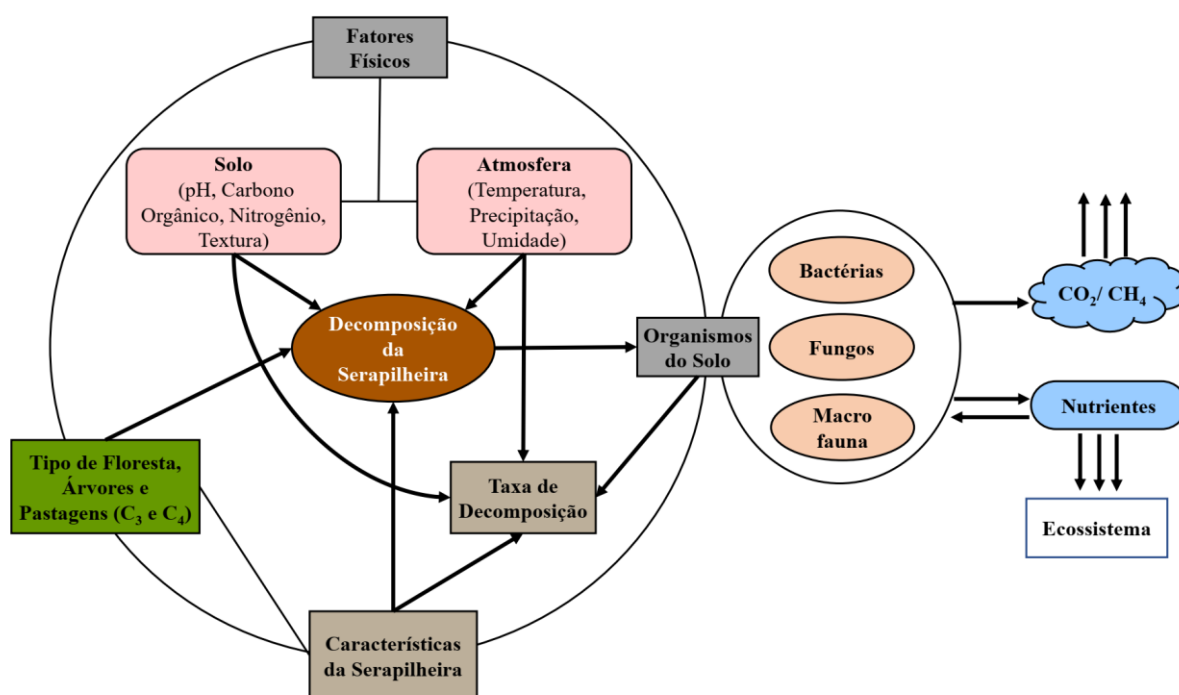


Figura 2. Representação esquemática dos fatores que afetam a decomposição da serapilheira. Redesenhado de Krishna e Mohan (2017).

A degradação da serapilheira pode ser influenciada por diversos fatores físicos, químicos e biológicos (KRISHNA e MOHAN; 2017). Alguns fatores como, inclusão de leguminosa no sistema (KOHMANN et al., 2019), utilização de adubos nitrogenados (DUBEUX et al., 2006b; APOLINÁRIO et al., 2014), aumento na taxa de lotação podem aumentar a taxa de decomposição (DUBEUX et al., 2006b).

A presença de fungos, bactérias, micro e macro invertebrados podem influenciar a taxa de decomposição, sendo esses influenciados pelas condições climáticas, como temperatura e precipitação (KRISHNA e MOHAN; 2017). Dentre os indicadores da qualidade da serapilheira que influenciam os processos de decomposição, a relação C:N é o mais utilizado (DUBEUX e SOLLENBERGER, 2020). Quando a relação C:N é superior a 30, processos de imobilização dos nutrientes ocorrem, enquanto abaixo desse valor, a mineralização é favorecida (WOLF e WAGNER, 2005; LAMBERS et al., 2008).

A inclusão de leguminosas em sistemas de gramíneas aceleram os processos de decomposição e mineralização dos nutrientes ao solo, por apresentarem menor relação C:N (REZENDE et al., 1999; GARCIA et al., 2013). Enquanto, serapilheira

produzida por gramíneas C_4 , geralmente apresentam baixo valor nutricional resultando em imobilização dos nutrientes, tais como N, P, Ca e Mg (THOMAS e ASAKAWA, 1993).

Para decomposições ao longo do tempo, outros indicadores de qualidade da serapilheira, como relação lignina:N ou lignina:NIDA, devem ser avaliados, pois somente a relação C:N não é considerada suficiente para explicar esses processos (APOLINÁRIO et al., 2013; DUBEUX e SOLLENBERGER, 2020). Altas relações de C:N, lignina:N, e NIDA em serapilheiras de gramíneas tropicais podem levar à imobilização dos nutrientes (VENDRAMINI et al., 2007), que poderiam estar disponíveis para o aproveitamento das forrageiras. O uso de adubos nitrogenados ou leguminosas consorciadas com gramíneas são considerados como boas opções para reduzir a imobilização desses nutrientes e melhorar a qualidade da serapilheira (VENDRAMINI et al., 2007; DUBEUX e SOLLENBERGER, 2020).

Aspectos relacionados à quantidade, qualidade e decomposição da serapilheira podem ser influenciados pela mudança nas condições climáticas durante o ano. Em situações de secas prolongadas a quantidade de serapilheira depositada aumenta e consequentemente seu desaparecimento diminui pela inibição do processo de decomposição (REZENDE et al., 1999). Isso porque os efeitos climáticos afetam principalmente o microclima e a microfauna existente no ecossistema das pastagens. Durante a transição do período da seca para as águas, geralmente há um aumento na massa de serapilheira seguido por uma diminuição devido à aceleração da taxa de decomposição (APOLINÁRIO et al., 2013).

A utilização de adubos nitrogenados podem afetar a relação dos indicadores da qualidade da serapilheira, influenciando assim no processo de decomposição bem como acelerando o retorno dos nutrientes ao solo (DUBEUX et al., 2006b), isso porque o N pode aumentar a decomposição da celulose e outros compostos solúveis (BERG e MATZNER, 1997). No entanto, o uso desses adubos, além de apresentar altos custos de produção, pode apresentar disponibilidade limitada dependendo da região (DUBEUX et al., 2011), além de não apresentar vantagens permanentes ao sistema de pastagens, sendo necessárias aplicações anuais.

Deste modo, a introdução de leguminosas ao sistema de gramíneas é considerada uma estratégia para melhorar a qualidade da serapilheira (DUBEUX e

SOLLENBERGER, 2020). A leguminosa pode ainda afetar a microfauna presente no solo, com isso, a dinâmica da decomposição pode ser alterada, bem como os processos de mineralização ou imobilização do N (CHAPMAN et al., 2013). O efeito do consórcio entre gramínea e leguminosa aumenta as taxas de decomposição da serapilheira, sendo que, com a inclusão de 50% de leguminosa no sistema pode ocorrer ainda a redução na imobilização de N, resultando no aumento da ciclagem total de nutrientes (SILVA et al., 2012).

2.6 Volatilização da amônia

A amônia (NH_3) é considerada um importante poluente atmosférico, responsável por impactos ambientais (BEUSEN et al., 2008), como a eutrofização e acidificação dos ecossistemas naturais (GRIZZETTI et al., 2011; GERBER et al., 2013), por meio da liberação de H^+ durante os processos de nitrificação (VELTHOF et al., 2011). No Setor Agropecuário, os adubos nitrogenados e a excreta animal (urina e fezes) são os principais responsáveis pelas perdas de N para o ambiente, ocasionando também prejuízos econômicos, pelo uso excessivo de compostos nitrogenados não aproveitados pelo sistema (solo, planta e animal).

Nos sistemas de pastagens, a deposição de urina e fezes pelos animais em pastejo são as principais fontes de volatilização de NH_3 (PHELAN et al., 2015). Estima-se que as perdas anuais globais de N via excretas representam quase 26 milhões de toneladas (GERBER et al., 2013) e os adubos nitrogenados 17 milhões de toneladas (XU et al., 2019). Dessa forma, levando em consideração o tamanho do rebanho brasileiro, estimado em cerca de 213,5 milhões de cabeças (IBGE, 2018), as perdas de N por meio da volatilização de NH_3 devem ser quantificadas pelo cenário agropecuário brasileiro e divulgados no inventário nacional de emissão de GEE.

Os fatores de emissão são as formas utilizadas para quantificar as emissões globais ou regionais de NH_3 , sendo definido como a porcentagem de N aplicado ao sistema volatilizado como NH_3 (XU et al., 2019). O Painel Intergovernamental sobre Mudanças Climáticas (IPCC, 2019) Tier 1 estima valores de volatilização de NH_3 de 30% (20 – 50%) para excretas (urina + fezes) e 15% (3 – 43%) para o adubo ureia.

Estudos anteriores demonstraram que a volatilização de NH_3 via urina pode variar entre 6 – 30% (MAJUMDAR et al., 2006; LESSA et al., 2014; CARDOSO et al., 2019b), fezes 1 – 7% (LESSA et al., 2014; CARDOSO et al., 2019b) e adubos nitrogenados entre 2 – 53% (MORAIS et al. 2013; SUTER et al., 2013). Essas grandes variações são explicadas por fatores climáticos, como precipitação, temperatura, velocidade do vento, radiação solar. Por fatores ligados à condição do solo, como temperatura, nitrificação (SOMMER e HUTCHINGS, 2001; ZAMAN et al., 2009; BURCHILL et al., 2017) e, principalmente, pH e umidade (NÚÑEZ et al., 2007). A dieta animal é responsável pelas excreções de N, bem como pela proporção que será excretado via urina e fezes (SATTER et al., 2002).

Baixos volumes de chuva (<8 mm) podem resultar no aumento da volatilização de NH_3 por meio da hidrólise da ureia, enquanto chuvas mais intensas (>18 mm) tendem a reduzir essa emissão devido à infiltração do N no solo seco (ENGEL et al., 2011). Outro fator que está relacionado às condições do clima é a mudança na temperatura. Elevações na temperatura provocam aumento na volatilização de NH_3 , enquanto em baixas temperaturas, pela inibição da hidrólise da ureia e dos processos de nitrificação, resultam em menores volatilizações (WHITEHEAD e RAISTRICK, 1991). Além dos fatores relacionados ao ambiente, o tipo de alimento consumido pelo animal (gramíneas, leguminosas ou concentrados) e a quantidade de água ingerida podem afetar na quantidade de N excretado (HRISTOV et al., 2013; BURCHILL et al., 2017), influenciando assim na volatilização de NH_3 .

Quando a urina, fezes ou adubos nitrogenados são depositados no solo, micro-organismos atuam sobre o N acelerando o mecanismo de volatilização da NH_3 (WACHENDORF; JOERGENSEN, 2011). O N excretado na urina ocorre entre 43 a 70% na forma de ureia (CARDOSO et al., 2019a). Outros compostos nitrogenados, como creatinina, ácido hipúrico e derivados de purina (alantoína, ácido úrico, xantina e hipoxantina) podem ser encontrados na urina (GARDINER et al., 2018; CARDOSO et al., 2019a). Nas fezes, o N pode ser excretado na forma de proteínas indigestíveis, proteína endógena, proteína microbiana indigestível e nitrogênio amoniacal (SATTER et al., 2002, PRADOS et al., 2016).

O processo de volatilização da NH_3 ocorre primeiramente pela hidrólise da ureia à carbonato de amônio nos solos, que se decompõe a dióxido de carbono (CO_2)

e amônio (NH_4^+) (BOLAN et al., 2004), essa reação é catalisada pela enzima urease, que está presente no solo, nas raízes, e na excreta animal (JARVIS e PAIN, 1990; WHITEHEAD, 1995; SAGGAR et al., 2013) (Figura 3). A volatilização de NH_3 nas fezes seguem os mesmos processos da urina, como uma elevação no pH da superfície de volatilização, formação de NH_4^+ durante a fase líquida, durante a fase gasosa, ocorre uma transição de equilíbrio entre NH_4^+ em NH_3 , e por fim, a emissão para a atmosfera por meio dos espaços porosos do solo (LAUBACH et al., 2013).

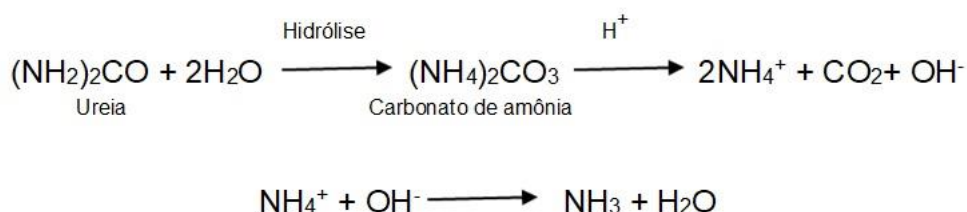


Figura 3. Amônia volatilizada pela hidrólise da ureia. Adaptado de Núñez et al., (2007).

Em relação ao uso de adubos nitrogenados, como a ureia [$\text{CO}(\text{NH}_2)_2$], sulfato de amônio [$(\text{NH}_4)_2\text{SO}_4$], nitrato de amônio (NH_4NO_3), nitrato de potássio (KNO_3) e nitrato de cálcio [$\text{Ca}(\text{NO}_3)_2$] diferenças em suas estruturas, quantidade de N (45%, 21%, 34%, 13% e 15% de N, respectivamente), parcelamento das doses e momento da aplicação (presença de chuva ou não) influenciam na volatilização de NH_3 (HE et al., 1999; HASLER et al., 2015). Embora a ureia seja o adubo nitrogenado que apresente menor custo por quilograma de nitrogênio (BOURSCHEIDT et al., 2019), perdas de N por volatilização de NH_3 podem atingir mais de 50% (ROCHETTE et al., 2009; MORAIS et al., 2013). Em contrapartida, adubos que contenham nitrato em sua composição podem resultar em maiores perdas de N por emissão de N_2O durante o processo de desnitrificação (HARRISON and WEBB, 2001).

Perdas de N de 20, 17 e 14% por volatilização de NH_3 foram encontradas para ureia, ureia recoberta com cobre e boro, e ureia tratada com inibidor de urease NBPT [N-(n-butil) tiofosfórico triamida], respectivamente, enquanto perdas abaixo de 1% foram encontradas para fontes não amídicas (nitrato de amônio, fusão de 75% de nitrato de amônio e 25% de sulfato de amônio, e mistura física de 75% de sulfato de amônio e 25% de nitrato de amônio), quando aplicados sobre a palha da cana (OTTO et al., 2017).

O uso de inibidores de urease e de nitrificação em adubos nitrogenados tem sido investigado como estratégias na mitigação de GEE. Resultados positivos são encontrados na literatura para mitigação de N₂O, por outro lado, esses inibidores podem aumentar significativamente a volatilização da NH₃ (KIM et al., 2012; LAM et al., 2016). Embora a NH₃ não seja considerada um GEE, ela atua como fonte secundária na produção de N₂O (MARTIKAINEN, 1985; ZAMAN et al., 2009). Dessa forma, a escolha de manejo de pasto que reduzam a aplicação dos adubos nitrogenados, como no caso a utilização de sistemas consorciados, torna-se importante para manter a produção de forragem para os animais de maneira mais sustentável.

Diante do exposto, com esta tese pretende-se verificar o uso do amendoim forrageiro (*Arachis pinto* cv. Amarillo) em consórcio com capim-marandu (*Urochloa brizantha* cv. 'Marandu') como estratégia de substituição ao uso do adubo nitrogenado ureia em sistemas de produção de forragem. Apresentando as seguintes hipóteses:

1) A dose de 150 kg N ha⁻¹ (parcelada igualmente em três aplicações) fornecerá aporte de nitrogênio suficiente para aumentar a produção de massa de forragem em relação ao pasto sem adubação, bem como a quantidade de material depositado como serapilheira. No entanto, maiores excreções de nitrogênio serão observadas para as novilhas mantidas nesse sistema adubado, o que resultará em maior volatilização de NH₃ para a atmosfera.

2) A inclusão do amendoim forrageiro em consórcio com o capim-marandu apresentará resultados semelhantes ao sistema de pastagem adubada, devido à fixação do nitrogênio atmosférico, promovendo melhor retorno de nutriente via serapilheira. Com a inclusão da leguminosa na dieta das novilhas, espera-se melhor aproveitamento do nitrogênio oriundo da forragem com a redução nas perdas via urina e fezes, reduzindo a volatilização de NH₃ em relação aos sistemas adubados com nitrogênio.

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CAPÍTULO 2 – Canopy characteristics of grazed N-fertilized palisadegrass or during establishment of palisadegrass-forage peanut mixtures¹

ABSTRACT

Palisadegrass [*Urochloa brizantha* (Hochst. ex A. Rich.) R. D. Webster cv. ‘Marandu’] is widely used in Brazil and typically managed with little or no N fertilizer, leading to pasture decline. The relationship between beef price and fertilizer cost does not favor fertilizer use in pastures. Legume inclusion is an alternative to add N. This study evaluated herbage responses to N inputs and vegetation types during establishment, under rotational stocking. Treatments included palisadegrass-forage peanut (*Arachis pintoii* Krapov. & W.C. Greg.) mixture (mixed), unfertilized palisadegrass (control), and palisadegrass fertilized with 150 kg N ha⁻¹ yr⁻¹ (fertilized). Treatments were applied for two growing seasons with five grazing cycles (GC) evaluations. Response variables included herbage mass, herbage accumulation, morphological components, total aboveground N (TAGN), and contribution of biological N₂ fixation (BNF). Pre-grazing herbage mass was greater for fertilized palisadegrass [5830 kg dry matter (DM) ha⁻¹] than for the palisadegrass-forage peanut mixture (3880 kg DM ha⁻¹), while the unfertilized palisadegrass (4400 kg DM ha⁻¹) did not differ from the mixed pasture. Nitrogen fertilizer increased leaf mass (2470 kg DM ha⁻¹) compared with the control and mixed treatments (1700 and 1310 kg DM ha⁻¹, respectively). The %Nd_f for forage peanut ranged from 79 to 85% and BNF ranged from 0.7 to 5.5 kg N ha⁻¹ cycle⁻¹. Overall, benefits from peanut were minimal because legume percentage was <10%. Beginning a grazing cycle before 95% canopy light interception or imposing a shorter post-grazing stubble height may aid in reducing shading to the decumbent-growing forage peanut when planted with palisadegrass.

¹Este capítulo corresponde ao artigo científico submetido nas normas da revista Crop Science e encontra-se em avaliação para publicação.

INTRODUCTION

In Brazil, grasses of the genus *Urochloa* cover an estimated 80 million ha. ‘Marandu’ palisadegrass [*Urochloa brizantha* (Hochst. ex A. Rich.) R. D. Webster cv. syn. *Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf.], the most used palisadegrass cultivar, is estimated to cover 56% of these area (Silva et al., 2016). Low-input systems prevail in Brazilian livestock operations, so limestone, gypsum, and chemical fertilizer applications are rare. The combination of low soil fertility, lack of chemical fertilization, and overgrazing results in pasture degradation or pasture decline (Boddey et al., 2004).

Nitrogen is a key element to improve pasture productivity (Sollenberger et al., 2009) and many warm-season C_4 grasses respond positively to N fertilization increasing herbage accumulation and improving nutritive value. However, this response varies with the environment, species, and management practices. Herbage accumulation of palisadegrass increased with increasing N fertilizer application from 90 to 270 kg ha⁻¹, but there was a diminished increase in herbage accumulation per unit of N applied, as the amount of N fertilizer increased (Delevatti et al., 2019). In this case, tripling the amount of N applied increased herbage accumulation only 1.18 times and increased potential for negative environmental impacts. Nitrogen fertilizer is manufactured using fossil fuel, which gives it a significant carbon footprint (Gerber et al., 2013). Furthermore, N fertilizer may be lost as nitric oxide (NO also called nitrogen oxide), nitrogen dioxide (NO₂), nitrous oxide (N₂O), di-nitrogen (N₂), ammonia (NH₃), or via nitrate (NO₃⁻) leaching (Zaman et al., 2009; Zaman et al., 2012). These processes may contribute to climate change (e.g., N₂O emission) or increase nitrate concentration in surface water bodies and groundwater.

Integrating legumes into grazing systems is an alternative to reduce the negative environmental impacts caused by N fertilization (Muir et al., 2014). Legumes add N to the

system via symbiotic biological dinitrogen fixation (BNF) between legume and rhizobium bacteria in root nodules (Sprent, 2007). In addition to reduced negative environmental impacts, legumes can also reduce production costs compared with N fertilizer (Muir et al. 2011; Muir et al., 2014).

The use of grass-legume mixed pastures in tropical regions is less commonplace than in temperate regions. In North America, mixing grasses and legumes is a more common practice (Williams et al., 1991), but it is still currently being investigated (Santos et al., 2018; Jaramillo et al., 2018). In Brazil, few studies have evaluated grass-legume mixtures under grazing. Challenges to legume use have been attributed to poor establishment and persistence compared with grasses (Muir et al., 2011; Alencar et al., 2018; Tamele et al., 2018). Forage peanut (*Arachis pinto* Krapov. & W.C. Greg. cv. Amarillo) is a legume that has a good potential to grow in mixtures in Brazil because it persists well when mixed with fast-growing grasses such as *Urochloa* (Hernandez et al., 1995; Andrade et al., 2012). A stoloniferous and prostrate growth habit makes the forage peanut ideal for grazing because its meristems are protected from grazing animals (Andrade et al., 2005). In tropical conditions, Tamele et al. (2018) reported that palisadegrass managed at a 20-cm canopy height improved forage peanut establishment in mixed systems. However, further research is necessary to determine if N from forage peanut growing in mixture with a grass is able to replace synthetic N fertilizer, especially during the establishment period.

We hypothesized that during the establishment of forage peanut with non-N fertilized grass the herbage characteristics such as herbage accumulation, green herbage mass, leaf mass, and leaf:stem ratio would be improved through BNF compared with unfertilized grass in a monoculture. We also hypothesized that mixed swards would have similar herbage accumulation compared to N fertilized swards. However, a low presence of forage peanut in

the present study occurred during the establishment period. We decided to continue the experiment to test if a low amount of forage peanut mixture with palisadegrass would result in any contribution to the mixed system. Therefore, this study evaluated the performance of palisadegrass under three sward management strategies (unfertilized, fertilized with 150 kg N ha⁻¹ yr⁻¹, or mixed with forage peanut), aiming to identify how different N inputs may affect pasture responses during the establishment period.

MATERIALS AND METHODS

Experimental Site

This study was conducted at Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil (21°14'05" S, 48°17'09" W; 615 m above sea level) during two growing seasons (November 2016 to March 2017 and December 2017 to April 2018). The soil at the experimental site is classified as an Oxisol (USDA, 2014). Soil samples (0- to 20-cm depth) collected in 2014, 2017, and 2018 indicated the following means: pH (CaCl₂) 5.3; organic matter 32.4 g kg⁻¹; cation exchange capacity 74.8 mmolc dm⁻³; P (ion-exchange resin extraction method) 10.9 mg dm⁻³; Mehlich-1 extractable Ca 28.3 mmolc dm⁻³; Mehlich-1 extractable Mg 9.7 mmolc dm⁻³; Mehlich-1 extractable K 4.2 mmolc dm⁻³; base saturation 561 g kg⁻¹, respectively (van Raij et al., 2001). Soil texture was 340 g kg⁻¹ sand, 140 g kg⁻¹ silt and 520 g kg⁻¹ clay (Claessen, 1997). Rainfall and mean temperature data from the experimental site were extracted from a dataset belonging to the Agrometeorology section of the Department of Exact Sciences (Fig. 1).

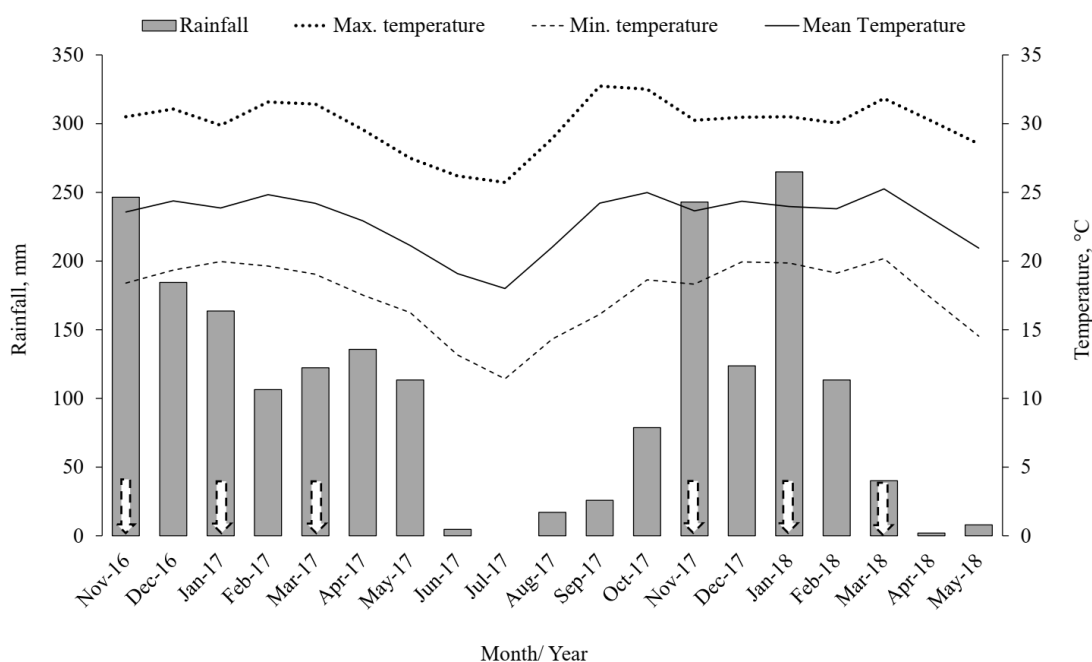


Fig. 1. Monthly rainfall and monthly mean air temperatures (maximum, mean, and minimum) from November 2016 to May 2018 in Jaboticabal, Brazil. Arrows indicate the nitrogen application ($150 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, split in three equal applications) in the fertilized treatment.

Treatments and Experimental Design

There were three treatments (sward management strategies, SM) with four replications, arranged in a completely randomized design with plot size of $20 \times 60 \text{ m}$. Treatments were 1) control - ‘Marandu’ palisadegrass in a monoculture, unfertilized (without N fertilizer or legume); 2) fertilized - palisadegrass fertilized with $150 \text{ kg N ha}^{-1} \text{ year}^{-1}$ (urea), split into three equal applications; and 3) mixed - palisadegrass mixed with forage peanut cv. ‘Amarillo’ and no N fertilizer. These treatments were evaluated for two growing seasons with five grazing cycle (GC) evaluations within each growing season: 19-30 Nov. 2016 and 4-15 Dec. 2017 (GC1), 10-21 Dec. 2016 and 3-14 Jan. 2018 (GC2), 9-20 Jan. 2017 and 5-16 Feb. 2018 (GC3), 6-17 Feb. 2017 and 12-23 Mar. 2018 (GC4), and 13-24 Mar. 2017 and 23 Apr.-4 May 2018 (GC5). Nitrogen was applied to the fertilized treatment in November, January, and March each growing season (50 kg N ha^{-1} per application).

Forage peanut presents a slow establishment when mixed with aggressive-growing grasses, however, over time this legume is able to compete and enhance its contribution in mixed system (Argel and Pizarro, 1992). The low presence of forage peanut during the establishment period occurred in the present study. Nevertheless, we decided to continue the experiment to test if a small amount of forage peanut mixture with grass resulted in any contribution to the mixed system.

Plot Establishment and Management

In March 2014, existent 'Tifton 85' bermudagrass (*Cynodon* spp.) was sprayed with 2880 g a.i. ha^{-1} of glyphosate [isopropylamine salt of N- (phosphonomethyl) glycine] in order to eradicate the existing vegetation. In October 2014, glyphosate plus 402 g a.i. ha^{-1} of 2,4-D amine (2,4-dichlorophenoxyacetic acid) were applied, and subsequently, a crusher and chopper (Tritton, model 1800) was used to remove dead residue. Grass monoculture was seeded subsequently with a row spacing of 0.45 m and a seeding rate of 10 kg ha^{-1} of pure live seed using a no-till seed drill (Semeato, model SHM 11-13). In the mixed treatment, forage peanut was simultaneously sown between rows of palisadegrass at a seeding rate of 8 kg ha^{-1} of pure live seeds using a seed drill (Jumil, model 2640 PD Exacta). After the seeding, all paddocks received 60 kg P, 20 kg S, 50 g Mo, 1 kg Cu and 1 kg B ha^{-1} , following recommendations of Werner et al. (1996). At the beginning of the growing season in both years, single superphosphate (40 kg P ha^{-1}) and potassium chloride (50 kg K ha^{-1}) were applied. The grass-legume mixed pastures were not fertilized with N.

Before seeding, the three treatments were randomized and allocated in 12 experimental units (1200 m^2 each). Each experimental unit was split into three paddocks of 400 m^2 . In September of each year, heifers were used to standardize the plots, and the paddocks were

defoliated using mob stocking (Allen et al., 2011). Grazing for all treatments initiated when one of the treatments reached 95% light interception (LI; Pereira et al., 2017) determined using the canopy analyzer LAI-2200 (LI-COR®, USA). The average of light interception was 95, 96, and 90% for control, fertilized, and mixed treatments, respectively. Usually, the first grazing cycle occurred approximately 40 d after start of the rainy season (November). Five grazing cycles occurred in each growing season with a rest period between them varying from 21 to 42 d because growth rates varied seasonally resulting in different amounts of time required to reach 95% LI. Grazing periods lasted 3 d, being 1 d for each paddock. Grazing ended on a given paddock when a stubble height of 15 cm was reached. A minimum of five crossbred heifers (311 ± 27 and 234 ± 19 kg, 2016/2017 and 2017/2018, respectively) was used for grazing. Additional heifers were added as needed to achieve the target stubble height in 1 d of grazing on a given paddock. The same animal group was used for the three paddocks within an experimental unit. A reserve palisadegrass pasture was used to keep the animals during the rest periods.

Response Variables

Herbage Characteristics

All samples were collected on the second paddock on the second grazing day. Herbage accumulation rate [kg of dry mass (DM) ha⁻¹ d⁻¹] was estimated as the difference between the pre-grazing herbage mass of the current grazing cycle and the post-grazing herbage mass of the previous grazing cycle (leaf + stem + dead mass was considered in this calculation), divided by the number of rest days between grazing cycles. Before putting the grazing animals on paddocks, the canopy height was estimated using a sward stick at 16 random points. The average canopy height was calculated and two samples representing the average herbage mass

were taken using 0.25-m² quadrats. The herbage mass was harvested at 2 cm from the ground level. A subsample taken from the herbage mass (fertilized and control treatment) was separated into leaf blade (leaf), stem plus sheath (stem), and dead material. When present, the inflorescence was included in the stem fraction. For the palisadegrass-forage peanut treatment, the entire sample was hand separated to measure the proportion of legume and grass and their respective morphologic components (leaf, stem, and dead mass). For calculation of pre-grazing herbage mass (kg DM ha⁻¹), all aboveground biomass (leaf + stem + dead mass) was considered; for the mixed treatment, total grass plus legume was considered. The green herbage mass (kg DM ha⁻¹) included the green leaf and stem without the dead mass. These fractions and the remaining non-separated herbage were oven-dried at 55°C for 72 h to calculate leaf:stem ratio (L:S). Canopy bulk density was calculated by dividing pre-grazing herbage mass by canopy height (kg DM ha⁻¹ cm⁻¹).

The ‘forage peanut selection index’ was the relationship between the percentage of forage peanut excreted in the dung divided by the percentage of forage peanut in the pasture botanical composition. We considered the percentage of legume excreted in the dung similar to the percentage of legume in the diet, because there is a positive linear regression ($r^2 = 0.97$) between $\delta^{13}\text{C}$ in the feed with $\delta^{13}\text{C}$ in the dung (Macedo et al., 2010). The dung sample was taken during the last 5 d of the grazing (total period of 12 d), and it was stored in plastic containers at -5 ± 2 °C until the last sampling. The dung was thoroughly mixed, and a sample was oven-dried at 55°C for 72 h. The proportion of legume in the forage was analyzed using the ^{13}C abundance technique. The proportion of C in the dung (%C_{legume}) derived from the legume was calculated using the equation indicated by Coates et al. (1987):

$$\%C_{\text{legume}} = \frac{100 \times (\delta^{13}\text{C}_{\text{mixed}} - \delta^{13}\text{C}_{\text{grass}})}{(\delta^{13}\text{C}_{\text{legume}} - \delta^{13}\text{C}_{\text{grass}})}$$

where $\delta^{13}\text{C}_{\text{mixed}}$ is the ^{13}C abundance in the dung from grass-legume mixed pasture, $\delta^{13}\text{C}_{\text{grass}}$ is the palisadegrass pasture and $\delta^{13}\text{C}_{\text{legume}}$ is the forage peanut pasture. $\delta^{13}\text{C}_{\text{grass}}$ and $\delta^{13}\text{C}_{\text{legume}}$ were determined from samples collected in the mixed pasture (Cantarutti et al., 2002).

Total Aboveground Nitrogen, Nitrogen Derived from the Atmosphere, and Biological N_2 Fixation by Forage Peanut

At the beginning of each grazing cycle, samples of total aboveground biomass of forage peanut and non- N_2 -fixing reference plants (weeds) including billygoat-weed (*Ageratum conyzoides* L.), beggar-ticks (*Bidens pilosa* L.), coat-buttons (*Tridax procumbens* L.), gomphrena-weed (*Gomphrena celosioides* Mart.), cockroach berry (*Solanum capsicoides* All.), and sanguinaria [*Alternanthera ficoidea* (L.) P. Beauv.] were collected from the same area. Different reference plants were collected due to the low number of weeds in the paddock. Samples were ground using a Wiley mill (Marconi, Ma680) to pass through a 1-mm stainless steel screen. After samples were roller milled for 16 h (Arnold and Schepers et al., 2004), total N concentration, abundance of ^{15}N and ^{13}C (‰) were analyzed using a mass spectrometer (model Delta V Advantage, Thermo Scientific) coupled to an elemental analyzer (model Flash 2000, Thermo Scientific) in the “John Day Stable Isotope Laboratory” at Embrapa Agrobiologia. The total aboveground N (TAGN, kg N ha⁻¹) was calculated multiplying the N concentration by the herbage accumulation of forage peanut (TAGN_{FP}, kg N ha⁻¹) and palisadegrass (TAGN_{PG}, kg N ha⁻¹). The percentage of N derived from the atmosphere (%Ndfa) for forage peanut was calculated using the equation described by Shearer and Kohl (1986):

$$\%Ndfa = \left(\frac{\delta^{15}\text{N}_{\text{reference}} - \delta^{15}\text{N}_{\text{legume}}}{\delta^{15}\text{N}_{\text{reference}} - \text{B}} \right) \times 100$$

where $\delta^{15}\text{N}_{\text{reference}}$ is the $\delta^{15}\text{N}$ value detected in a reference plant (non-N fixing) growing in the same soil at the same grazing cycle in the palisadegrass-forage peanut paddock, $\delta^{15}\text{N}_{\text{legume}}$ is the $\delta^{15}\text{N}$ abundance of the forage peanut, and B is the $\delta^{15}\text{N}$ value of the legume obtained from dinitrogen fixation. We used the B value of -1.35‰ reported by Unkovich et al. (2008) for *Arachis hypogea* L. in Brazil. The $\delta^{15}\text{N}$ from reference plants ranged from 4.4 to $5.9\text{‰} \pm 0.6\text{‰}$, with values changing with sampling date. The total contribution of N from BNF was calculated by multiplying TAGN_{FP} by the $\% \text{Nd}_{\text{fa}}$ (Unkovich et al., 2008).

Statistical Analysis

All data were analyzed using the MIXED procedure of the SAS statistical package (SAS Inst. Inc., Cary, NC, USA). For the analysis of variance of palisadegrass, SM, GC and their interaction were fixed effects. The paddock (SM Year) were random effects. The GC were used as repeated measures. The Akaike information criterion (AIC) was used to choose the best covariance structure. Satterthwaite approximation was used to determine the denominator degrees of freedom for the test of fixed effects. The LSMEANS were compared using the PDIFF procedure by Fisher's protected least significant difference (LSD) test at 5% probability. For the analysis of variance of forage peanut, the GC was the fixed effect. The paddock (GC Year) were the random effects. The Satterthwaite approximation was used to determine the denominator degrees of freedom for the test of fixed effects. The LSMEANS were compared using the PDIFF procedure by Fisher's protected least significant difference (LSD) test at 5% probability.

RESULTS AND DISCUSSION

Herbage Characteristics

Nitrogen fertilizer enhanced pre-grazing herbage mass by 25 and 33% ($P = 0.03$) and green herbage mass by 32 and 41% ($P = 0.01$), compared with unfertilized palisadegrass and palisadegrass-forage peanut mixture, respectively (Table 1). Nitrogen is generally the most limiting nutrient for plant growth, enhances tillering (Silva et al., 2013) and is a major component of the chlorophyll molecule (Chapman and Barreto, 1997). In similar conditions to those of this study, Delevatti et al. (2019) reported an increasing linear effect of N fertilization (0, 90, 180, and 270 kg N ha⁻¹ year⁻¹) on palisadegrass herbage mass. In the Brazilian Cerrado, N fertilization (100 kg N ha⁻¹) increased palisadegrass herbage accumulation by 22% compared with unfertilized palisadegrass (Dupas et al., 2016). There was no difference for pre-grazing herbage mass and green herbage mass between the unfertilized palisadegrass and palisadegrass-forage peanut mixture, indicating that inclusion of the legume in the system did not negatively affect palisadegrass growth. We hypothesized that forage peanut could fix atmospheric N and supply N to palisadegrass. However, the N input by the forage peanut in our study was not sufficient to promote growth of palisadegrass in the mixed system similar to the N-fertilized system.

Table 1. Pre-grazing herbage mass and herbage characteristics of palisadegrass in monoculture, palisadegrass fertilized with N (150 kg ha⁻¹, split in three equal applications), or forage peanut–palisadegrass mixed swards.

Item ‡	Sward management strategies (SM)			SE#	Grazing cycle (GC)					SE	P-value		
	Control	Fertilized	Mixed¶		1	2	3	4	5		SM	GC	SM×GC
PHM, kg DM ha ⁻¹	4400ab†	5830a	3880b	490	4380	4460	4650	5060	4960	400	0.03	0.66	0.52
GHM, kg DM ha ⁻¹	3030b	4440a	2600b	400	3370	3190	3450	3770	3020	330	0.01	0.19	0.83
HAR, kg DM ha ⁻¹ d ⁻¹	67	83	51	14	NA	81a	56ab	78a	52b	13	0.32	0.04	0.10
Leaf:stem ratio	1.7	1.5	1.7	0.2	2.2a	1.4b	1.5b	1.5b	1.5b	0.1	0.78	0.01	0.15

† Means not followed by the same letter are different according to the PDIFF procedure by LSD test ($P < 0.05$).

‡ PHM= pre-grazing herbage mass, GHM = green herbage mass, HAR = herbage accumulation rate.

NA = Herbage accumulation rate was evaluated between grazing cycles. The grazing cycle 1 (November 2016 and December 2017) was not evaluated, because the stubble mass after the standardization grazing in September was not performed.

¶ Values of palisadegrass with forage peanut.

SE – Standard error.

There was no difference between SM for herbage accumulation rate, however, GC influenced the response (Table 1). Ribeiro et al. (2008) reported a greater herbage accumulation rate when a bermudagrass-forage peanut mixture was fertilized with N compared with an unfertilized mixture; however, the herbage accumulation rates were similar when 100 or 200 kg N ha⁻¹ was applied in the mixed systems. The authors suggested the dry season affected the legume recovery in the rainy season. In our study, the forage peanut in the botanical composition decreased in the last grazing cycle, by the drought conditions at the beginning of the transition of rainy season to dry season. Although we did not evaluate responses during the dry season, we noticed that the low rainfall during the dry season affected the presence of forage peanut. When the rainy season began, the forage peanut initiated growth, however, its recovery was slow. This may partially explain the lesser response of mixed systems in our study. In the same region, Delevatti et al. (2019) found a linear effect of N fertilizer rate on herbage accumulation rate of palisadegrass. However, in our study, the herbage accumulation rate of the N-fertilized treatment did not differ from mixed or unfertilized systems (Table 1). Sanchez et al., (2018) did not find difference in the herbage accumulation rate using bermudagrass in a monoculture or mixed with forage peanut (28 vs. 19 kg ha⁻¹ d⁻¹), however, the authors observed difference between months due to limited rainfall and temperature. The weather conditions in different grazing cycles affected herbage accumulation. The values decreased from 81 to 52 kg DM ha⁻¹ d⁻¹ from GC2 to GC5, respectively. Average rainfall during the rest period between GC was 95, 60, 116, and 42 mm (for GC1 to GC2, GC2 to GC3, GC3 to GC4, and GC4 to GC5, respectively). Probably this pattern contributed to increasing the herbage accumulation rate in the GC2 and GC4, whereas no differences were observed in GC3 and GC5 due to less favorable temperature and rainfall (Fig. 1).

There was no difference between SM for L:S ratio of green herbage mass, however, GC influenced the response (Table 1, $P < 0.01$). Greater L:S ratio was observed only in the GC1. The experimental period started two months after the grazing standardization, when the treatments reached 95% light interception. Probably the two months of rest period resulted in greater leaf mass. When the grazing cycles started, the rainfall increased, and the rest period reduced (~32 days), resulting in low leaf mass (Table 2). There was a reduction of the L:S ratio after the GC1. It was expected that N-fertilized grasses would show greater L:S ratio by the increase in the leaf number and/or leaf elongation (Santana et al., 2016). However, the N fertilization increased the leaf proportion as well as the stem proportion (Table 2). Alencar et al. (2018) did not find a difference for L:S ratio for the palisadegrass in a mixture or a monoculture. Similar results were found in this study.

Morphological Components of Palisadegrass

Canopy height, leaf mass, and stem mass of palisadegrass at pre-grazing event were affected by SM and GC (Table 2, $P < 0.05$). Canopy height was greater in the N fertilized treatment (38 cm) than in the control (26 cm) and in the mixed sward (27 cm). Canopy height at 95% light interception was not constant for the SM studied. Therefore, canopy height can be a better strategy to compare monoculture and mixed pastures in future studies, at lower cost. Pereira et al. (2017) reported light interception between 90 and 95% with canopy height ranging from 26 to 32 cm for palisadegrass-forage peanut mixtures as the best grazing management to provide greater herbage accumulation rate, especially leaves. Brougham (1958) indicated that 90 to 95% light interception leads to greatest herbage accumulation rates, especially in monoculture or mixed pastures. Canopy height control in mixed systems is important because there are two different plant species competing for light in the same area. Tamele et al. (2018) in a harvesting

study (weekly harvests) reported proportions of forage peanut ranging from 11 to 17% when the canopy height of palisadegrass was 30 cm; the optimum canopy height to increase the proportion of forage peanut was 20 cm. In the current study, the increase of canopy height might have decreased the proportion of legume, leaf mass, and subsequently $TAGN_{FP}$ and BNF. In pastures sampled seven years after planting, Gomes et al. (2018) found canopy height of palisadegrass in mixed system ranging between 25 (for 90% LI) and 30 cm (for 95% LI) without inhibiting the growth of the forage peanut in rotational stocking. Canopy height decreased in the GC5. This response may have occurred by the drought conditions at the beginning of the transition season (rainy to dry season), resulting in low green herbage mass (leaf and stem) and great dead mass in the GC5. This variation in canopy height suggests that there was no correlation between canopy height and light interception with the change from the rainy to the dry season. Canopy height is affected by management strategy, forage species, and season; therefore, the same LI may result in different canopy heights, mainly due to changes in plant architecture resulted from change in those factors. In our study, we used 95% LI target to start the grazing period, and the canopy height changed among GC (ranged from 26 to 33 cm). Pereira et al. (2017) reported similar results among evaluation period of palisadegrass-forage peanut (26 to 33 cm).

Table 2. Morphological components (leaf, stem, and dead mass), canopy bulk density, and canopy height of palisadegrass in monoculture, palisadegrass fertilized with N (150 kg ha⁻¹, split in three equal applications), or forage peanut–palisadegrass mixed swards.

Item‡	Sward management strategies (SM)			SE#	Grazing cycles (GC)					SE	P- value		
	Control	Fertilized	Mixed¶		1	2	3	4	5		SM	GC	SM×GC
CH, cm	26b†	38a	27b	3	33a	30a	32 ^a	31a	26b	2	0.02	0.01	0.08
LM, kg DM ha ⁻¹	1700b	2470a	1310b	130	2230a	1670bc	1910ab	1950ab	1390c	130	0.01	0.01	0.62
SM, kg DM ha ⁻¹	1350ab	1960a	1020b	270	1090b	1320ab	1510ab	1770a	1540ab	210	0.05	0.03	0.79
DM, kg DM ha ⁻¹	1360	1390	1330	140	1010c	1360b	1190bc	1290bc	1940a	130	0.95	0.01	0.01
CD, kg DM ha ⁻¹ cm ⁻¹	170	160	138	10	136c	154abc	143bc	167ab	180a	11	0.07	0.02	0.16

† Means not followed by the same letter are different according to the PDIFF procedure adjusted by LSD test ($P < 0.05$).

‡ CH = canopy height, LM = leaf mass, SM = stem mass, DM = dead mass, CD = canopy bulk density.

¶ Values for only palisadegrass (without forage peanut).

SE – Standard error.

Leaf mass of palisadegrass for the N-fertilized treatment was greater than the control and mixed (2470 vs. 1700 and 1310 kg DM ha⁻¹, respectively). The N is linked with plant growth and tissue turnover. An explanation is N fertilization may increase the leaf number and/or leaf elongation (Santana et al., 2016). Pereira et al. (2015) reported increases of 25% in leaf elongation rate in palisadegrass fertilized with 150 kg N ha⁻¹ when compared to unfertilized pastures during the summer. Fagundes et al. (2006) found a linear increase in leaf elongation rate for palisadegrass as N rates increased (75, 150, 225 and 300 kg N ha⁻¹ yr⁻¹). Silva et al. (2016) reported that N fertilization from 50 to 250 kg N ha⁻¹ yr⁻¹ resulted in an increase in leaf proportion of Mulato II brachiariagrass hybrid (*U. ruziziensis* × *U. decumbens* × *U. brizantha*) from 22 to 25%. Leaf mass was greatest in GC1 and lowest in GC5 (2230 vs. 1390 kg DM ha⁻¹, respectively). The experimental period started two months after the grazing standardization. Probably this rest period resulted in greater leaf mass. When the grazing cycles started, the rainfall increased, and the rest period reduced (~32 days), resulting in low leaf mass (Table 2). The shortest rest period was between GC1 and GC2 (21 and 30 days), probably it was responsible for the low leaf mass in the GC2, whereas the GC5 increased the rest period (35 and 42 days) due to limited rainfall, resulting in great dead mass and low leaf mass.

Palisadegrass stem mass for the N-fertilized treatment was greater than the mixed treatment (1960 vs. 1020 kg DM ha⁻¹, respectively), which did not differ from the control treatment (1350 kg DM ha⁻¹). Nitrogen fertilization might have enhanced regeneration of the tiller population by increasing tiller appearance rate and tiller death; those factors can vary with season due to the weather conditions, such as temperature and rainfall (Caminha et al., 2010). In addition, the N fertilized treatment always reached first the 95% light interception than the other treatments, and this may have resulted in a competition for light between tillers, the plant will elongate the stem, especially in search of luminosity for the younger leaves (Santana et al.,

2016). The lowest stem mass was in GC1 and the greatest in GC4 (1090 vs. 1770 kg DM ha⁻¹). Average rainfall during the rest period between GC3 and GC4 was 116 mm. During the rainy season, the tiller may have increased the population or elongation or become heavier to sustain more weight (Santana et al., 2016). The stem is an important fraction for the tropical grasses because it is responsible for plant growth and leaf support to enhance light interception (Fagundes et al., 2006).

There was a SM $\times$ GC interaction on the dead herbage mass of palisadegrass (Table 2, $P < 0.01$). Within of GC, there was difference between SM in the GC2 and GC5 (Fig. 2a). The N fertilization reduced the dead herbage mass in the GC2, however, in the GC5 the N fertilized increased the herbage dead mass. The mixed and control treatments were not different from each other in both GC. Within of SM, there was difference between GC for the control, fertilized, and mixed treatments (Fig. 2b). Lower dead herbage mass was observed in the GC1, GC3, and GC4 for the fertilized, control, and mixed treatment, respectively. Whereas, greater dead herbage mass was observed in the GC2 and GC4 for the control treatment, and in the GC5 for the fertilized and mixed treatments. Dead herbage mass of palisadegrass ranged from 990 to 1590 kg DM ha⁻¹, 640 to 2670 kg DM ha⁻¹, and 1010 to 1700 kg DM ha⁻¹ for control, fertilized, and mixed treatment, respectively. These responses may have occurred because the N fertilizer was applied in November, January, and March. The N fertilization combined with favorable weather conditions (temperature and rainfall) likely promoted greater green mass and reduced the dead herbage mass in the GC2, while the N fertilization in March and drought conditions at the beginning of the transition season may have increased the dead herbage mass in the GC5. Nitrogen fertilization likely affected maturity stage in the last GC period, and may have hastened flowering stem elongation and leaf death compared to other treatments. Nitrogen fertilization might have enhanced regeneration of the tiller population by increasing tiller

appearance rate and tiller death; those factors can vary with season due to the weather conditions, such as temperature and rainfall (Caminha et al., 2010). Pereira et al. (2015) reported increases of 22% in leaf appearance rate in palisadegrass fertilized with 150 kg N ha⁻¹ when compared with unfertilized pastures during the summer. This acceleration in leaf appearance rate may be responsible for increasing the dead material later in the season in our study. In a tropical environment, Gomes et al. (2018) reported that dead herbage mass for palisadegrass-forage peanut mixtures did not change between grazing frequencies (initiated at 100, 95, and 90% LI or grazed every 42 d) and seasons, with an average of 2332 kg ha⁻¹. Our study showed lower dead mass for the mixed system, and there was a difference in the variable among CG, and it was affected mainly by the weather conditions and soil nutrients. Similar pattern occurred to control treatment.

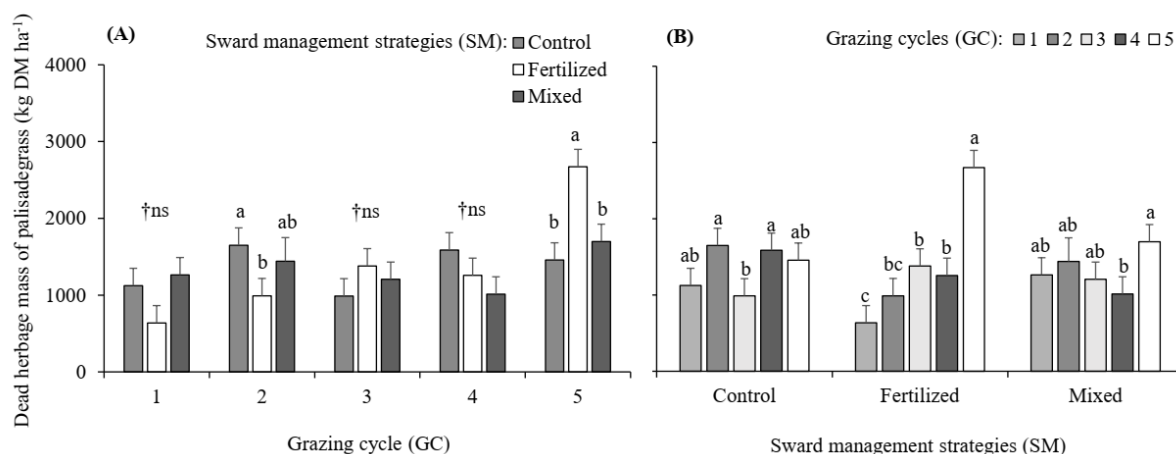


Fig. 2. Sward management strategies × GC interaction effect on dead herbage mass of palisadegrass ($P < 0.01$, SE = 230). Error bars refer to the SE. †Non-significant. Data are means across 2 yr, and four replicates ($n = 8$). Different lowercase letter within GC (A) and SM (B) are different according to the PDIFF procedure by LSD test ($P < 0.05$).

Canopy bulk density varied with GC ($P = 0.02$) for palisadegrass. Means ranged from 136 to 180 kg DM ha⁻¹ cm⁻¹ (Table 2). The lowest means were observed in GC1, and the

greatest was in GC5. These differences are reflections of the herbage mass and the canopy height promoted by stem elongation of the palisadegrass during the GC, which are influenced by the weather conditions (Fig. 1). As rainfall decreased, the stem proportion increased and consequently canopy bulk density increased. Many elements may influence the canopy bulk density including plant species (C_4 and C_3), pasture management (defoliation intensity), and weather conditions (Sollenberger and Burns, 2001).

Morphological Components of Forage Peanut

There were differences between GC for leaf and stem mass, but no differences were found for the dead mass of forage peanut. The dead herbage mass was consistently low, ranging from 4 to 22 kg DM ha⁻¹ (Table 3, $P = 0.26$). In the dry season, leaf loss typically occurs for forage peanut (Gomes et al., 2018), whereas during the rainy season there are greater growth rates due to greater leaf appearance rate and elongation (Pereira et al., 2017), with less death occurring. In this study, the leaf mass of forage peanut ranged from 21 to 197 kg DM ha⁻¹ (Table 3, $P = 0.03$), with the lowest value found in GC5. In these GC in southeastern Brazil the transition period between the rainy and dry season begins. Stem mass of forage peanut ranged from 20 to 181 kg DM ha⁻¹ (Table 3, $P = 0.05$). Gomes et al. (2018) reported that stem mass for forage peanut did not change with grazing frequency treatments or seasons. Our study showed a reduction in stem mass in GC5, probably due to rainfall reduction and the beginning of the dry season. Our values are lower (113 vs. 766 kg ha⁻¹, respectively) than those reported by Gomes et al. (2018). However, their pasture was established 7 yr before the experimental period. In this study, the forage peanut was not fully established yet because the experimental period began 2 yr after seeding. Pre-grazing herbage mass of forage peanut ranged from 249 to 390 kg DM ha⁻¹, decreasing in the GC5 to 51 kg DM ha⁻¹. Pereira et al. (2020) found 493 kg DM

ha⁻¹ of forage peanut mixed with palisadegrass in the rainy season, and the mixed treatment resulted in a liveweight gain per ha of 28.7% greater than in the palisadegrass N fertilized with 120 kg N ha⁻¹.

Table 3. Morphological components (leaf, stem and dead mass), canopy bulk density, and canopy height of forage peanut. The response variables are means across four replicates, and 2 yr (n = 8).

Item‡	Grazing Cycle (GC)					SE#	P value
	1	2	3	4	5		
CH, cm	9	8	9	8	6	1	0.48
LM, kg DM ha ⁻¹	152a	197a	153a	111a	21b	70	0.03
SM, kg DM ha ⁻¹	107a	181a	129a	129a	20b	65	0.05
DM, kg DM ha ⁻¹	4	12	22	9	10	6	0.26
CD, kg DM ha ⁻¹ cm ⁻¹	27	44	29	27	11	10	0.39

Means not followed by the same letter are different according to the PDIFF procedure by LSD test ($P < 0.05$).

‡ CH = canopy height, LM = leaf mass, SM = stem mass, DM = dead mass, CD = canopy bulk density.

SE – Standard error.

There was no difference between GC for canopy height, and canopy bulk density of forage peanut (Table 3, $P \geq 0.05$). Canopy height ranged from 6 to 9 cm, and the canopy bulk density ranged from 17 to 44 kg DM ha⁻¹ cm⁻¹. Canopy bulk density in the current study was lower than reported previously for different cultivars of rhizoma peanut (*Arachis glabrata* Benth.) in monoculture (126 – 270 kg DM ha⁻¹ cm⁻¹; Mullenix et al., 2016). Those authors suggested that canopy bulk density was influenced by cultivar, season, and grazing management (Mullenix et al., 2016). High canopy bulk density may reduce the number of tongue sweeps, bite volume, and bite weight (Burns and Sollenberger, 2002). It can limit the selection of leaf and, consequently, reduces animal performance (Newman et al., 2002).

Botanical Composition

The botanical composition differed between GC (Fig. 3a; $P < 0.01$, $SE = 3$). Weed presence was minimal, and the data are not reported. The greatest proportion of palisadegrass (98%) was found in GC5, and the lowest proportion (91%) was found in GC2. The greatest proportion of forage peanut (9%) was found in GC2, and the lowest proportion (2%) was found in GC5. The botanical composition in GC1, GC3, and GC4 averaged 93 and 7% of palisadegrass and forage peanut, respectively. The presence of forage peanut in the mixed system declined as the rainy season ended. Warm-season C_4 grasses are typically more competitive than legumes, shifting the balance in mixed swards towards the grass (Alencar et al., 2018). This competition can reduce the persistence of forage peanut in mixed systems. Grass establishment, growth, and time to recover after grazing tend to be faster than for legumes (Muir et al., 2011). In the Brazilian tropics, Tamele et al. (2018) reported a reduction in the proportion of forage peanut with increased canopy height (10, 20, 30, and 40 cm) of palisadegrass in a harvesting study (weekly harvests). The authors concluded that the canopy height of 20 cm provided the best proportion of legume (19 – 38%) in a mixed sward, without compromising the grass growth. The increase in grass canopy height can stimulate vertical growth of forage peanut, reducing the number of stolons and decreasing legume proportion in the mixed system. These changes in growth habit of forage peanut may limit its ability to compete with palisadegrass in mixed stands. In addition, the lower proportion of the legume in the botanical composition may be caused by the slow establishment (Alencar et al., 2018). Forage peanut growth may have been limited by shading from palisadegrass (Tamele et al., 2018). Santos et al. (2018) reported 57% rhizoma peanut in mixtures with bahiagrass 4 yr after seeding. It is possible that forage peanut available for use in Brazil is less vigorous during the establishment phase than rhizoma peanut. Studies in Florida USA show good results in the use of rhizoma peanut cultivars in mixed

systems (Jaramillo et al., 2018; Santos et al., 2018). *Arachis pintoi* has great tolerance to grazing, mainly because the growing points are protected from grazing animals, unlike most of the tropical legume species. Thus, it is possible to maintain residual leaf area even when the plant is subjected to continuous stocking and intense defoliation (Lima et al., 2003).

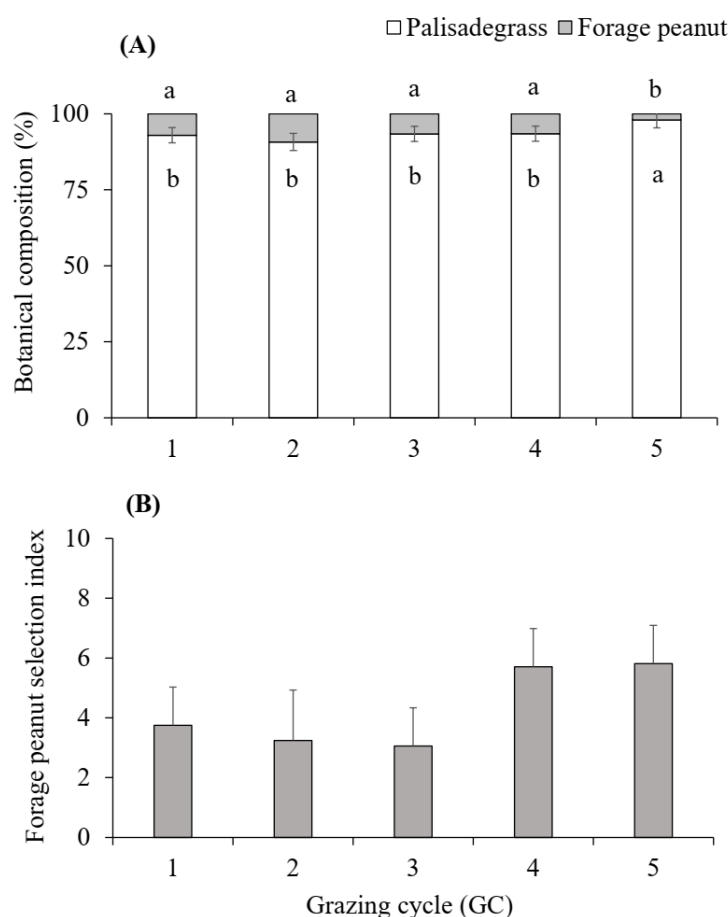


Fig. 3. Botanical composition of palisadegrass and forage peanut (A) affected by GC ($P < 0.01$, SE = 3) and forage peanut selection index (B) ($P = 0.41$, SE = 1). Error bars refer to the SE. The response variables are means across four replicates, and 2 yr ($n = 8$). Different lowercase letters are different between GC, according to the PDIFF procedure by LSD test ($P < 0.05$).

Although the proportion of forage peanut in the sward was low (mean 6% of forage peanut), the grazing animals selected it, resulting in a greater proportion in the diet. The forage peanut selection index for animals ranged from 2 to 6 from GC1 to GC5 (Fig. 3b). These results demonstrate the importance of including forage peanut in a mixed system.

Total Aboveground Nitrogen, Nitrogen Derived from the Atmosphere, and Biological N₂ Fixation by Forage Peanut

The total aboveground N of forage peanut (TAGN_{FP}) was affected by GC (Table 4; $P = 0.05$), ranging from 1 to 7 kg N ha⁻¹ cycle⁻¹. Greater N in herbage mass was present in GC1 and did not differ from GC2 and GC3. The low herbage accumulation of forage peanut resulted in low TAGN_{FP}. Leaf mass (Table 3) and presence of forage peanut in the botanical composition (Fig 3a) were greater in GC1 to GC4. Total herbage accumulation of forage peanut for five GC was 613 kg DM ha⁻¹, resulting in a TAGN_{FP} of 17 kg N ha⁻¹ (GC1 to GC5). The TAGN_{FP} decreased when the forage peanut in the herbage accumulation decreased (GC4 and GC5). Jaramillo et al. (2018) reported values of 0.7 kg N ha⁻¹ harvest⁻¹ for forage peanut mixed with bahiagrass. Santos et al. (2018) reported TAGN for rhizoma peanut-bahiagrass mixtures of 50 kg N ha⁻¹. In our study, the forage peanut demonstrated low potential to compete with the palisadegrass, due to slow legume establishment. The nutrient competition between grass and legume, the lack of N fertilization in the first years of establishment of the mixed system, and the slow establishment of forage peanut may have resulted in low herbage mass of forage peanut. During the establishment period of grass-legume mixed pastures, N fertilization can be used considering that legumes have limited capability to fix atmospheric N₂ during initial developmental stages (Andrade et al., 2014). Two weeks after germination, Alencar et al. (2018) fertilized a palisadegrass-forage peanut mixture with 30 kg N ha⁻¹, but the amount of N fixation was small (~ 4.0 kg ha⁻¹) due to the slow growth of the forage peanut.

Table 4. Total aboveground N of palisadegrass ($TAGN_{PG}$) and forage peanut ($TAGN_{FP}$), nitrogen derived from the atmosphere (%Ndfa) and biological N_2 fixation (BNF) of forage peanut. The response variables are means across four replicates, and 2 yr ($n = 8$).

Item	Grazing cycle					SE‡	P-value
	1	2	3	4	5		
<i>Palisadegrass</i>							
TAGN _{PG} , kg N ha ⁻¹ cycle ⁻¹	31a†	19b	18b	13b	19ab	4	0.05
<i>Forage peanut</i>							
TAGN _{FP} , kg N ha ⁻¹ cycle ⁻¹	7a	3ab	4ab	2b	1b	2	0.05
%Ndfa	79	81	82	82	85	2	0.14
BNF, kg N ha ⁻¹ cycle ⁻¹	5.5a	2.4ab	3.2ab	1.7b	0.7b	1.8	0.05

† Means not followed by the same letter are different according to the PDIFF procedure by LSD test ($P < 0.05$).

‡ SE – Standard error.

The total aboveground N of palisadegrass ($TAGN_{PG}$) was affected by GC (Table 4; $P = 0.04$). The $TAGN_{PG}$ ranged from 13 to 31 kg N ha⁻¹ cycle⁻¹. Greatest N aboveground was found in GC1 and the lowest in GC4. The difference in the rainfall and temperature (Fig. 1) can explain the differences found between GC. Weather conditions influence herbage accumulation and N concentration in the grass and legume. Total herbage accumulation of palisadegrass for five GC was 9785 kg DM ha⁻¹ (GC1 to GC5), resulting in a $TAGN_{PG}$ of 100 kg N ha⁻¹. The low proportion of legume in the mixed system may have resulted in low TAGN. The fibrous root systems of the C₄ plants compete for superficial moisture and soil nutrients when compared with taproot systems of C₃ plants (Muir et al., 2011). Santos et al. (2018) found values of TAGN for bahiagrass mixed with rhizoma peanut of 6 kg N ha⁻¹ harvest⁻¹.

There was a SM × GC effect on TAGN (Fig. 4; $P < 0.01$, SE = 12). Within of GC, there was difference between SM in the CG1 and GC4 (Fig. 4a). The N fertilization increased the TAGN in both GC. The mixed treatment was the lowest TAGN in the GC1, while in the GC4 did not differ from control treatment. Within of SM, there was difference between GC for the control and fertilized treatments, while the mixed treatment did not differ between GC (Fig. 4b). The TAGN ranged from 22 to 76 kg N ha⁻¹ cycle⁻¹, 34 to 113 kg N ha⁻¹ cycle⁻¹, and 15 to

38 kg N ha⁻¹ cycle⁻¹ for control, fertilized and mixed treatment, respectively. Greater N yields were found in GC1, being greater than other GC for both the control and fertilized treatments. Considering that TAGN is a product of forage N concentration and herbage accumulation, the N fertilizer directly affected results (Fig. 1). There was no difference for TAGN between GC when forage peanut and palisadegrass were analyzed together. Santos et al. (2018) reported TAGN ranging from 6 to 40 kg N ha⁻¹ harvest⁻¹ for rhizoma peanut-bahiagrass mixture and bahiagrass fertilized with 90 kg N ha⁻¹ harvest⁻¹, respectively.

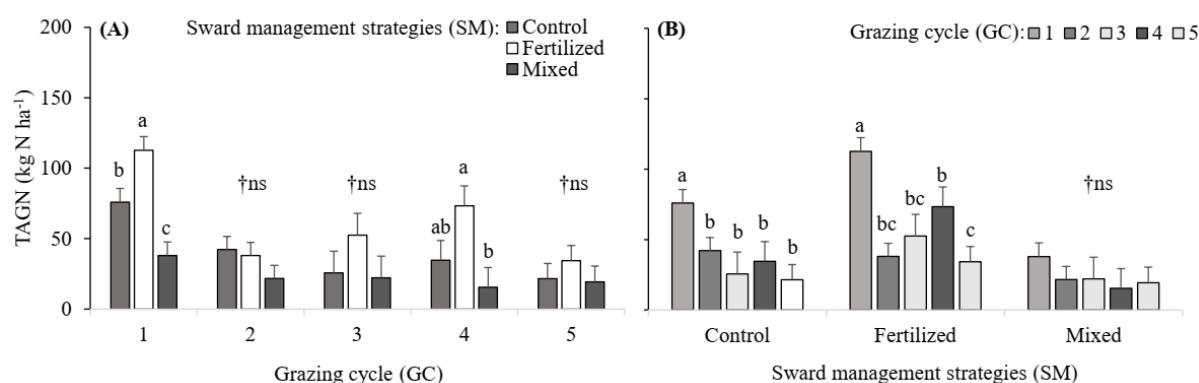


Fig. 4. Total aboveground N (TAGN) of palisadegrass without N inclusion (control), fertilized with 150 kg N ha⁻¹ yr⁻¹ (fertilized), and mixed with forage peanut (mixed) as affected by SM × GC interaction ($P < 0.01$, SE = 12). Error bars refer to the SE. †Non-significant. Data are means across four replicates, and 2 yr ($n = 8$). Different lowercase letter within GC (A) and SM (B) are different according to the PDIFF procedure by LSD test ($P < 0.05$).

There was no difference between GC for %Ndfa for forage peanut (Table 4; $P = 0.14$). Values ranged from 79 to 85% in GC1 to GC5. Our study found values similar to the literature. Alencar et al. (2018) reported a Ndfa of ~80% for forage peanut. Dubeux et al. (2017) reported Ndfa ranging from 67 to 96% for seven rhizoma peanut cultivars. Santos et al. (2018) found %Ndfa ranging from 60 to 82% for rhizoma peanut in a monoculture or mixed with bahiagrass.

The authors observed that legumes in the mixture presented similar %Ndfa compared with legumes in monocultures.

The BNF varied with GC (Table 4; $P = 0.05$), and this was likely linked to the reduction of the leaves. The aboveground biomass is associated with the reduction of BNF (Carvalho et al., 2019). Greater BNF was found in GC1, probably because of better weather conditions for plant growth (Fig. 1). The total amount of N_2 fixation was $13.5 \text{ kg N ha}^{-1}$ (GC1 to GC5). These results are relatively small due to the reduced growth of forage peanut ($< 10\%$ of the legume in the botanical composition) in the mixed system and mainly due to slow establishment. The proportion of 20 to 30% legume in the botanical composition of pastures may fix 50 to $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Andrade et al., 2014). Jaramillo et al. (2018) found BNF ranging from 0 to $2.7 \text{ kg N ha}^{-1} \text{ harvest}^{-1}$ for forage peanut-bahiagrass mixtures. Alencar et al. (2018) reported BNF of 3.4 kg N ha^{-1} for forage peanut. The authors concluded that during the legume establishment period, there was a reduced BNF due to the slow growth of the legume. A similar pattern was observed in our study. Other authors reported greater BNF with the legume inclusion in mixed systems (Santos et al., 2018); however, some factors, such as difference between peanut cultivars, establishment time, and persistence may have influenced the proportion of forage peanut in the pasture, and this was reflected in the BNF. Additionally, in our study the pasture was seeded in 2014 and the trial performed in November 2016 to March 2017 and December 2017 to April 2018, 2 yr after the seeding. In the mixed management, we did not fertilize with N, and the competition for nutrients between legume and grass may have resulted in low results for both forages. Forage peanut mixed with palisadegrass is considered promising; however, significant competition that negatively affects forage peanut establishment occurs in early stages following planting (Alencar et al., 2018). Further studies need to evaluate seeding

Arachis with machinery and applying N fertilization during the early stages after planting to determine if legume establishment rate can improve.

SUMMARY AND CONCLUSION

The small amount of forage peanut in the mixed system presented similar results to the unfertilized palisadegrass in most of the response variables during the establishment period. Botanical composition fluctuated according to the grazing cycle, with forage peanut disappearance at the beginning of the transition from rainy to dry season. Biological N₂ fixation and TAGN_{FP} decreased with the reduction of leaf mass, which was affected by the different weather conditions during the season. In this study, the forage peanut inclusion was not enough to guarantee better results of the mixed system when compared to the N fertilized palisadegrass. The N fertilization enhanced the pre-grazing herbage mass and green herbage mass of palisadegrass, especially the proportion of leaves. However, as rainfall decreased, the dead mass increased.

The evaluation during the grazing cycle demonstrated the growth pattern of the grass in a tropical environment as well as forage peanut. However, the legume was less tolerant to lack of water with the beginning of the transition to the dry season. Different management strategies such as the beginning of the grazing cycle before 95% of light interception or lower stubble heights should be employed, considering that legumes (C₃) and grasses (C₄) require different management mainly during the establishment period.

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CAPÍTULO 3 – Nitrogen fertilizer and environmental conditions affect litter deposition in warm-climate grasslands during pasture establishment¹

ABSTRACT

In warm-climate grasslands, litter deposition and decomposition are the main sources of nutrient cycling. The application of nitrogen (N) fertilizer or the inclusion of a legume in such grasslands can improve the litter characteristics and its chemical composition. This study evaluated how N supply of palisadegrass [*Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster] pastures affect litter characteristics two years after establishment. Treatments were palisadegrass fertilized or not fertilized with N or *Arachis pintoii* mixture. Response variables included existing litter mass, litter deposition rate, litter decomposition rate, and litter chemical composition. Nitrogen fertilization increased existing litter mass and litter deposition rate compared with grass monoculture or legume-grass mixtures. Both variables were affected by weather conditions, with low litter deposition rate and existing litter mass in the grazing cycle with poor rainfall (GC4). Nitrogen fertilized palisadegrass had greater litter N concentration [$7.9 \pm 0.4 \text{ g kg}^{-1}$ organic matter (OM)] resulting in lower C:N ratio (34 ± 3) than in monoculture or legume-grass mixtures (44 and 58 ± 3 , respectively). Results indicated that N fertilization in palisadegrass increased litter deposition rate as well as existing litter mass and litter N concentration. After two years of establishment, the proportion of forage peanut in the litter was still low, reducing the benefits of legume inclusion to enhance litter nutrient cycling in grassland ecosystems. Further studies will be necessary to evaluate the grass-legume mixture in the longer term.

¹Este capítulo corresponde ao artigo científico submetido nas normas da revista Ecosystems e encontra-se em avaliação para publicação.

INTRODUCTION

In grassland ecosystems, litter is a key driving factor affecting nutrient cycling, herbage production, and soil organic carbon (Krishna and Mohan 2017). Warm-climate grasses are widely used in tropical regions; however, these pastures typically lack N fertilization, mainly due to the high prices of synthetic fertilizers and/or limited availability, depending on the region (Dubeux and others 2007; Dubeux and others 2011). Warm-climate C₄-grass pastures typically have greater herbage production and lower nutritive value when compared with C₃ grass ones, resulting in low-quality litter for the former. Inefficient nutrient cycling resulting from poor-quality litter and overgrazing are major drivers of pasture decline (Boddey and others 2004).

Nitrogen fertilization is a strategy to enhance warm-climate grass productivity, improving growth and forage nutritive value (Sollenberger and others 2009). Greater forage nutritive value results in better quality litter returning to the soil. Nitrogen fertilization on warm-climate grass may increase litter decomposition rate (Dubeux and others 2006; Liu and others 2011). Nonetheless, excessive use of synthetic fertilizers or animal manure may result in environmental contamination (Woodard and others 2003). Nitrogen fertilization presents short-lived results and must be applied every year for continuing benefits. On the other hand, the inclusion of legume in tropical grass pastures may enhance litter quality, improving soil fertility and forage nutritive value (Cantarutti and others 2002). A study in Florida assessed bahiagrass-rhizoma peanut (*Arachis glabrata* Benth.) mixtures in two proportions (33 and 67% of legume), monoculture of legume, unfertilized grass, and nitrogen fertilized grass, the authors concluded that mixed pastures were the most efficient in returning nutrients to the system (Kohmann and others 2019). Furthermore, mixed litter may provoke changes in soil microorganisms and affect dynamics of litter decomposition (Chapman and others 2013).

Litter decomposition can be the most important pathway of nutrient return in warm-climate grasslands, especially at low stocking rates. The decomposition process can be affected by litter quality, N input, soil microorganisms, stocking rate, and weather conditions. Litter quality is defined by the physical properties and chemical composition, especially the concentrations of N, P, C, lignin, and polyphenols and their ratios (Thomas and Asakawa 1993; Cotrufo and others 1994). Seasonal variations result in changes in temperature and rainfall throughout the year. These variations affect the soil environment such as the presence of fauna and activity of microorganisms which may influence litter decomposition rate (Krishna and Mohan 2017). Dry periods in the summer increase the amount of litter deposition in tropical grasses, and the litter disappearance decreases due to reduced decomposition during such periods (Rezende and others 1999).

Litter is not only a source of nutrient, but it also acts in soil temperature control, soil cover, soil moisture, erosion protection, and soil fertility (Heal and others 1997). Litter with low C:N ratio reduces problems related to pasture decline. Although the effect of stocking rate and N application are known on litter disappearance (Dubeux and others 2006; Liu and others 2011; Apolinário and others 2014), there is limited information on the effect of legume inclusion into *Urochloa* pastures in tropical regions. The objective of this study was to quantify the effects of sward management strategies including (1) grazing management of palisadegrass in a monoculture, (2) mixture with forage peanut (*Arachis pinto* cv. Amarillo) and, (3) nitrogen fertilization two years after seeding, across five grazing cycles, on litter deposition and decomposition rate.

MATERIALS AND METHODS

Experimental Site

This study was conducted at Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil (21°14'05" S e 48°17'09" W; 615 m above sea level) during two growing seasons (November 2016 to March 2017 and December 2017 to April 2018). The soil at the experimental site is classified as an Oxisol (USDA, 2014). Soil samples (0 to 20-cm depth) collected in 2014, 2017, and 2018 showed the following mean composition: pH (CaCl₂) 5.3; organic matter 32.4 g kg⁻¹; cation exchange capacity 74.8 mmolc dm⁻³; P (ion-exchange resin extraction method) 10.9 mg dm⁻³; Mehlich-1 extractable Ca 28.3 mmolc dm⁻³; Mehlich-1 extractable Mg 9.7 mmolc dm⁻³; Mehlich-1 extractable K 4.2 mmolc dm⁻³; base saturation 561 g kg⁻¹, respectively (van Raij et al., 2001). Soil texture was 340 g kg⁻¹ sand, 140 g kg⁻¹ silt and 520 g kg⁻¹ clay (Claessen, 1997). Rainfall and average temperature data of the present study were obtained from a dataset belonging to the Agrometeorology section of the Department of Exact Sciences (Figure 1).

Treatments and Experimental design

There were three treatments with four replications, arranged in a completely randomized design. Treatments (TR) were: palisadegrass in a monoculture without N fertilizer or legume (unfertilized); palisadegrass fertilized with 150 kg N ha⁻¹ yr⁻¹, source urea, split into three equal applications (fertilized); and palisadegrass-forage peanut mixture no N fertilizer (mixed). These treatments were evaluated for two growing seasons after seeding with five grazing cycles (GC) within each growing season: 19-30 Nov. 2016 and 4-15 Dec. 2017 (GC1), 10-21 Dec. 2016 and 3-14 Jan. 2018 (GC2), 9-20 Jan. 2017 and 5-16 Feb. 2018 (GC3), 6-17 Feb. 2017 and 12-23 Mar. 2018 (GC4), and 13-24 Mar. 2017 and 23 Apr.-4 May 2018 (GC5). The pastures were not grazed during the dry season (May to October). Nitrogen was applied to the fertilized

treatment in November, January and March each growing season (50 kg N ha⁻¹ per application; Figure 1).

Forage peanut presents a slow establishment when mixed with aggressive-growing grasses, however, over time this legume is able to compete and enhance its contribution in mixed system (Argel and Pizarro, 1992). The low presence of forage peanut during the establishment period occurred in the present study. Nevertheless, we decided to continue the experiment to test if a small amount of forage peanut mixture with grass resulted in any contribution to the mixed system.

Plot Establishment and Grazing Management

In March 2014, existent 'Tifton 85' bermudagrass (*Cynodon* spp.) was sprayed with 2,880 g a.i. ha⁻¹ of glyphosate [isopropylamine salt of N- (phosphonomethyl) glycine] in order to eradicate this grass. In October 2014, glyphosate plus 402 g a.i. ha⁻¹ of 2,4-D amine (2,4-dichlorophenoxyacetic acid) were applied, and subsequently, a crusher and chopper (Tritton, model 1800) was used to lower the stubble height. Grass monoculture was seeded subsequently with a row spacing of 0.45 m and a seeding rate of 10 kg ha⁻¹ of pure live seed using a no-till seed drill (Semeato, model SHM 11-13). In the mixed treatment, forage peanut was simultaneously sown between rows of palisadegrass at a seeding rate of 8 kg ha⁻¹ of pure live seeds using a seed drill (Jumil, model 2640 PD Exacta). After seeding, all paddocks received 60 kg P, 20 kg S, 50 g Mo, 1 kg Cu and 1 kg B ha⁻¹, following recommendations by Werner et al. (1996). At the beginning of the growing season in both years, single superphosphate (40 kg P ha⁻¹) and potassium chloride (50 kg K ha⁻¹) were applied. The grass-legume mixed pastures were not fertilized with N.

Before seeding, the three treatments were randomized and allocated in 12 experimental units (1200 m² each). Each experimental unit was split into three paddocks of 400 m². In September of each year, heifers were used to standardize the plots, and the paddocks were defoliated using mob stocking (Allen et al., 2011). Grazing for all treatments initiated when one of the treatments reached 95% light interception (LI; Pereira et al., 2017) determined using the canopy analyzer LAI-2200 (LI-COR®, USA). The average of light interception was 95, 96, and 90% to control, fertilized, and mixed treatment, respectively. Usually, the first grazing cycle occurred approximately 40 d after start of the rainy season (November). Five grazing cycles occurred in each year with a rest period between them varying from 21 to 42 d. Grazing periods lasted 3 d, 1 d for each paddock. Grazing ended on a given paddock when a stubble height of 15 cm was reached. A minimum of five crossbred heifers (311 ± 27 and 234 ± 19 kg, 2016/2017 and 2017/2018, respectively) was used for grazing. Additional heifers were added as needed to achieve the target stubble height in 1 d of grazing on a given paddock. The same animal group was used for the three paddocks within an experimental unit. A reserve palisadegrass pasture was used to keep the animals during the rest periods.

Existing Litter, Litter Deposition, and Litter Decomposition

All samples were collected from the third paddock on the third grazing day. Before the animals started grazing, four quadrats (0.25-m²) were placed at random locations in each paddock. Dead plant material no longer attached to the plant on the soil surface inside the quadrat was hand-collected and oven-dried for >72 hours at 55 °C and weighed. This material was called existing litter mass. The area around the frame (~30 cm) was cleaned, removing all litter to avoid contamination of the next sampling by wind, rainfall or trampling of animals (Rezende and others 1999). The litter deposition was measured after 1 d of grazing, and each 14 d until the

next grazing cycle. The litter deposited on the soil surface was collected from the previously cleared area following the methodology described by Bruce and Ebersohn (1982) and Rezende and others (1999). This procedure was followed in each grazing cycle which ranged from 21 to 42 d; the period required for the palisadegrass to reach 95% LI. The material was dried at 55°C for >72 h. After the drying procedure and before weighing, both existing litter and deposited litter were sieved consecutively through 4- and 2-mm sieves to remove soil and then weighed. Samples were analyzed for dry matter (DM) by drying at 105°C for 16 h and OM by ashing at 500°C for 4 h (Moore and Mott, 1974). All data were expressed on organic matter (OM) basis to eliminate mineral particle effects on nutrient concentration. Litter deposition rate ($\text{kg OM ha}^{-1} \text{ d}^{-1}$) was obtained by dividing the total litter deposited between grazing cycles by the numbers of day to start the next grazing cycle (Rezende and others 1999; Apolinário and others 2013). Litter decomposition rate (constant k) was calculated based on equation 9 from Rezende and others (1999):

$$k = -\ln[(LE_0 + LD_N)/LE_0]/t_N \quad (1)$$

where LE_0 is the quantity of existing litter at day zero, LD_N is the litter deposited between consecutive samplings of existing litter, and t_N is the number of days between consecutive samplings of existing litter. In our study, the numbers of days between grazing cycles (or consecutive samplings of existing litter) were not fixed (varied from 21 to 42-d), because they depended of the number of the days needed to palisadegrass reach 95% light interception. Litter half-life ($t^{1/2}$) was calculated based on equation 10 from Rezende and others (1999):

$$t^{1/2} = \ln(2)/k \quad (2)$$

The proportion of the litter deposited after grazing (%) was calculated by dividing litter deposited after 1-d of grazing (kg DM ha^{-1}) by total litter deposited between grazing cycles (kg

DM ha⁻¹) multiplied by 100. This litter deposited after grazing was classified as the material lost due to grazing (i.e. trampling).

Litter Chemical Composition Analysis

Samples were ground using a Wiley mill (Marconi, Ma680) to pass through a 1-mm stainless steel screen. Samples were analyzed for DM by drying at 105°C for 16 h and OM by ashing at 500°C for 4 h (Moore and Mott, 1974). After samples were roller milled for 16 h (Arnold and Schepers 2004), the carbon (C), nitrogen (N), and the abundance of ¹³C (‰) were analyzed using a mass spectrometer (model Delta V Advantage, Thermo Scientific) coupled to an elemental analyzer (model Flash 2000, Thermo Scientific). All chemical composition results were expressed on an OM basis to eliminate mineral particle effects on nutrient concentration. Carbon:N ratio was obtained by dividing C concentration (g kg⁻¹ OM) by N (g kg⁻¹ OM).

The abundance of ¹³C (‰) in the litter was analyzed using a mass spectrometer (model Delta V Advantage, Thermo Scientific) coupled to an elemental analyzer (model Flash 2000, Thermo Scientific) in the “John Day Stable Isotope Laboratory” at Embrapa Agrobiologia. The proportion of C in the litter derived from the legume (%C_{legume}) was calculated from the equation used by Cantarutti and others (2002):

$$\%C_{\text{legume}} = \frac{100 \times (\delta^{13}\text{C}_{\text{mixed}} - \delta^{13}\text{C}_{\text{grass}})}{(\delta^{13}\text{C}_{\text{legume}} - \delta^{13}\text{C}_{\text{grass}})} \quad (3)$$

where $\delta^{13}\text{C}_{\text{mixed}}$ is the ¹³C abundance in the litter from grass-legume mixed pasture, $\delta^{13}\text{C}_{\text{grass}}$ is the litter from palisadegrass and $\delta^{13}\text{C}_{\text{legume}}$ is the litter from forage peanut.

Statistical analysis

All data were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA) with Satterthwaite approximation to determine the

denominator degrees of freedom for the test of fixed effects. Paddock was the experimental unit, and treatment, grazing cycles and their interactions were included as fixed effects, and paddock(treatment years) were included as random effects. The grazing cycles were used as repeated measure. The Akaike information criterion was used to choose the best covariance structure. For analysis of forage peanut, the grazing cycle was the fixed effect, and years were included as random effects. The LSMEANS were compared using the PDIFF procedure by Fisher's protected least significant difference (LSD) test. Differences were declared significant at $p < 0.05$.

RESULTS

Average existing litter mass in unfertilized, fertilized, and mixed pastures were 1670, 2380, and 1470 kg ha⁻¹ OM, respectively. The N fertilizer increased existing litter mass, whereas legume inclusion did not differ from unfertilized palisadegrass (Table 1, $p = 0.004$). Weather conditions affected existing litter mass (Figure 2a, $p < 0.001$). At the beginning of the experiment, greater existing litter mass was registered in the GC1, declining in GC2 and GC3, with the lower existing litter mass in the GC4. With the onset of the dry season, existing litter mass started to increase again in the GC5. Treatments and grazing cycles affected the litter deposition rate (Table 1, Figure 2b). The N fertilizer increased litter deposition in relation to unfertilized and mixed treatments. These variables were also affected by weather conditions. Lower litter deposition rate was observed in GC4, whereas greater litter deposition was observed in GC1 and GC5, when the trial started and finished. Litter deposition rate ranged from 14 to 21 kg ha⁻¹ d⁻¹ OM across grazing cycles. Approximately 50% of the litter was deposited in the first day of occupation, and it was affected by weather variations across grazing

cycles (Figure 2c, $p < 0.0001$). Lower litter deposited after grazing was observed in GC4, whereas greater litter deposited after grazing was observed in GC2.

Relative decomposition rate, k , ranged from 0.014 to 0.016 $\text{g g}^{-1} \text{d}^{-1}$ OM across treatments (Table 1), it resulted in half-life of 49, 44 and 42 days to unfertilized, fertilized, and mixed treatment, respectively.

N fertilization increased litter N and reduced litter C:N ratio (Table 2, $p < 0.05$); the mixed treatment did not differ from unfertilized for litter N, however, presented greater C:N ratio. Greater litter N concentration was observed in the GC1, declining in the GC2 and GC3, these grazing cycles did not differ from GC4 and GC5 (figure 3a, $p = 0.032$). Litter C:N ratio was affected by weather and ranged from 43 to 48 across the grazing cycles (figure 3b, $p = 0.009$).

The ^{13}C abundance of *Arachis pinto* was -26.3 and -28.3‰ while for palisadegrass was -11.0 and 12.1‰, for growing seasons 2016/2017 and 2017/2018, respectively. The $\delta^{13}\text{C}$ in the mixed treatment ranged from -12.5 to -13.0‰, but it was not different across the grazing cycles ($p = 0.282$). These values were responsible for a proportion of 8.2% of the forage peanut (ranging between 6.2 to 9.6%) in the litter deposited in 14-day periods (Table 2).

DISCUSSION

Effect of treatments and grazing cycles on litter deposition

The existing litter mass is a function of litter deposition rate and litter decomposition rate (Rezende and others 1999). Differences observed in existing litter mass can be attributed primarily to differences in litter deposition rate, considering that litter decomposition rate was similar among treatments and grazing cycles (Table 1). Grasses promptly take up fertilizer N that is linked to processes such as renovation of tiller population, increase on tiller appearance rate, and tiller death (Caminha and others 2010; Silva and others 2013). Therefore, it is

expected that N-fertilized grasses will show greater values for existing litter mass and litter deposition rate. The mixed treatment presented similar existing litter mass to the unfertilized treatment. The low herbage mass of 4400 kg DM ha⁻¹ for unfertilized and 3880 kg DM ha⁻¹ for mixed vs. 5830 kg DM ha⁻¹ for fertilized (data not shown), in those treatments may have influenced the lower quantity of litter deposited as litter mass. Our findings are consistent with those of Kohmann and others (2018) that found in unfertilized warm-season grass a similar to the litter deposition rate in a grass-rhizoma peanut mixture. Probably in our study, the proportion of legume in the mixed system was not enough to significantly affect the existing litter mass ($8.2 \pm 2.3\%$ of forage peanut) due to a low N supply. The slow establishment and short growth habit of forage peanut reduce the ability of the legume to compete with grass in early stages after establishment (Alencar and others 2018). Although in our study the legume proportion was low in the mixed systems, over the years, the forage peanut tends to increase (5.9 ± 2.1 to $7.5 \pm 2.0\%$ of the legume in the botanical composition in 2016/2017 and 2017/2018, respectively). It seems likely that if the grazing of the palisadegrass was managed with shorter rest periods and more severe defoliation the proportion of forage peanut would have increased more rapidly (Homem and others 2019).

At the beginning of the experiment, greater existing litter mass was observed in the GC1 (2200 kg ha⁻¹ OM); A portion of the litter on the ground may have been residual from the previous dry season. The area in our study remained ungrazed from May to November in both years (approximately 7 months without grazing). This indicates a likely lag time in the litter decomposition and litter accumulation (Rezende and others 1999; Apolinário and others 2013). In addition, greater litter deposition was observed during the same period. Greater litter deposition, poor litter quality, and slower litter decomposition results in greater litter mass accumulation (Lambers and others 2008). Moreover, at the beginning of the growing season,

N fertilization, legume-N deposition or weather conditions were not enough to accelerate litter decomposition. The lowest mass of existing litter and litter deposition rate occurred in the GC4. This grazing cycle had the lowest rainfall in both years. Seasonal variation in rainfall and temperature, commonly found in warm-climate regions, changes the litter deposition rate (Apolinário and others 2013).

A fraction of the litter deposited is due to animal trampling and grazing losses (Apolinário and others 2013). This demonstrates the importance of the grazing animal for nutrient cycling in a grassland ecosystem. In our study, the stocking rate was adjusted to maintain similar herbage allowance among treatments. This may explain the absence of effect between treatments for litter deposition after grazing.

Stem mass (1290 ± 130 kg DM ha⁻¹; data not shown) and canopy heights (31 ± 2 cm; data not shown) of palisadegrass were greater during GC4. Plant architecture (canopy height and density) affect grazing behavior (Sollenberger and Burns 2001), affecting the amount of the litter deposition after grazing as a result. An increase in litter deposition rate, litter deposited after grazing, and existing litter mass was observed in GC5; the beginning of the dry season may have influenced the grass death and deposited as litter. The transition from rainy to dry season is often characterized by slower litter decomposition rate and greater deposition rate (Apolinário and others 2013).

Litter deposition was not constant over the days. Litter deposition after grazing (one day of grazing) represented 50% of total litter deposited between grazing cycles (21 to 42-d between grazing cycles). Probably this occurs by the intermittent stocking method used in our study. Deposition peaks may occur at the end to the beginning of the grazing cycle, due to leaf growth, new leaf appearance, and old leaf senescence.

Effect of treatments and grazing cycles on litter decomposition

In the present study, the litter decomposition rate was not affected by the N supply management. Previous studies found greater litter decomposition rates ranging from 0.038 to 0.089 g g⁻¹ d⁻¹ (Rezende and others 1999; Ramalho and others 2019). Abiotic factors (temperature, rainfall, and seasonal variations) may influence the existence of biotic factors (animal trampling, microbes and other soil fauna) that significantly affect decomposition rate (Krishna and Mohan 2017). Thus, it was a surprise that N fertilizer and legume inclusion did not affect litter decomposition rate in this study. However, the lack of effect of the nitrogen fertilization or legume inclusion has been reported in previous studies (Kohmann and others 2019; Ramalho and others 2019). In our study, a possible explanation is the management of nitrogen fertilization, which was split in three applications (November, January, and March). This split application was likely not enough to provide a difference between treatments, and the proportion of the legume in the litter was lower than 10% (Table 2). This proportion is unlikely to be enough to change the composition of the decomposer community (macro and microorganisms) or the litter quality, especially in the first year of mixed pasture establishment. Low legume proportion in warm-climate grass-legume mixtures was not enough to accelerate litter decomposition (Kohmann and others 2018), and the slow decomposition results in the building up of soil organic matter and soil nutrient stocks (Isaac and Nair 2005).

Litter of warm-season C₄ grasses often is poor in quality and may result in nutrient immobilization (Thomas and Asakawa, 1993) instead of mineralization. Mineralization is favored when C:N ratio is below 20, whereas immobilization is favored when C:N ratio is greater than 30 (Wolf and Wagner, 2005; Lambers and others 2008). However, our results for C:N ratio were above 30 (Table 2, Figure 3b); only for the N-fertilized treatment the C:N ratio was 34 ± 3. Nitrogen fertilization increased litter N concentration and decreased litter C:N ratio,

improving its quality, but this litter did not decompose faster than litter from unfertilized or mixed treatments, nor with the changing in the weather conditions (temperature or rainfall; Figure 1). In a previous study, Kohmann and others (2018) reported lower C:N ratio for bahiagrass-rhizoma peanut compared with unfertilized and fertilized bahiagrass at the end of the incubation period (128 d). Litter C:N ratio was not a complete indicator of decomposition processes and C and N linked to other components must also be analyzed.

Other indicators of litter quality include lignin, P, acid detergent insoluble N and polyphenols contents, and their ratios with C and N are often used as indicators (Thomas and Asakawa, 1993). Although in our study the lignin was not evaluated, previous study found lignin concentration of 26 g kg⁻¹ DM in palisadegrass and 43 g kg⁻¹ DM in forage peanut plant (Gomes and others 2018). Greater lignin concentration in forage peanut might explain the lack of effect on decomposition rate. In litter of bermudagrass-rhizoma peanut mixtures, Kohmann and others (2018) found values of lignin concentration ranging from 34 to 150 g kg⁻¹ OM during incubation (0 to 128d). Lignin is considered a recalcitrant compound (Six and others 2002) and increases during the decomposition process by the disappearance of labile litter components (Cotrufo and others 2015).

CONCLUSION

Nitrogen fertilization in palisadegrass pastures resulted in an increase of existing litter mass and litter deposition rate. The period of two years after seeding of mixed grass-legume pasture was insufficient to change litter characteristics and these results were similar to those recorded for the unfertilized grass. Seasonal variation between grazing cycles control the existing litter mass and litter deposition rate. The material deposited such as litter by losses after grazing events represented 50% of the total litter deposited between grazing cycles, the grazing activity

being responsible for most nutrient return to soil via litter. Different N inputs to warm-climate grasslands did not impact litter decomposition rate, resulting in an average of 45 days of litter half-life, even though litter N increased and litter C:N ratio was reduced for N-fertilized palisadegrass. The proportion of 10% of legume or less in grass-legume mixtures was not enough to affect the litter quality, litter decomposition rate, or litter existing mass, but other indicators and other legume proportions must be further investigated.

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Table 1. Existing litter, litter deposition, and decomposition of palisadegrass under three nitrogen supply.

	Treatments (TR) ¹			SE	<i>p</i> -value
	Unfertilized	Fertilized	Mixed		
Existing litter mass, kg ha ⁻¹ OM	1670b	2380a	1470b	180	0.004
Litter deposition rate, kg ha ⁻¹ OM	16b	21a	17b	1	0.022
Litter deposited after grazing, %	50	51	50	3	0.953
Decomposition rate (<i>k</i>), g g ⁻¹ d ⁻¹ OM	0.014	0.016	0.016	0.002	0.792

¹Unfertilized = palisadegrass without nitrogen fertilizer or legume; Fertilized = palisadegrass fertilized with urea (150 kg N ha⁻¹, split in three equal applications); Mixed = palisadegrass-forage peanut mixtures no N fertilizer. The response variables are means across four replicates, 2 yr, and five evaluations per year (n = 40).

Means not followed by the same letter are different according to the PDIFF procedure by LSD test (*p* < 0.05).

SE = Standard error.

Table 2. Chemical composition of deposited litter of palisadegrass three nitrogen supply.

	Treatments (TR)			SE	<i>p</i> -value
	Unfertilized	Fertilized	Mixed		
Litter N, g kg ⁻¹ OM	5.6b	7.9a	5.1b	0.4	0.001
Litter C:N ratio	44b	34c	58a	3	<0.0001
δ ¹³ C, ‰	-	-	-12.8	0.3	-
Forage peanut LD _{14d} , %	-	-	8.2	2.3	-

¹Unfertilized = palisadegrass without nitrogen fertilizer or legume; Fertilized = palisadegrass fertilized with urea (150 kg N ha⁻¹, split in three equal applications); Mixed = palisadegrass-forage peanut mixtures no N fertilizer. The response variables are means across four replicates, 2 yr, and five evaluations per year (n = 40).

Means not followed by the same letter are different according to the PDIFF procedure by LSD test (*p* < 0.05).

SE = standard error.

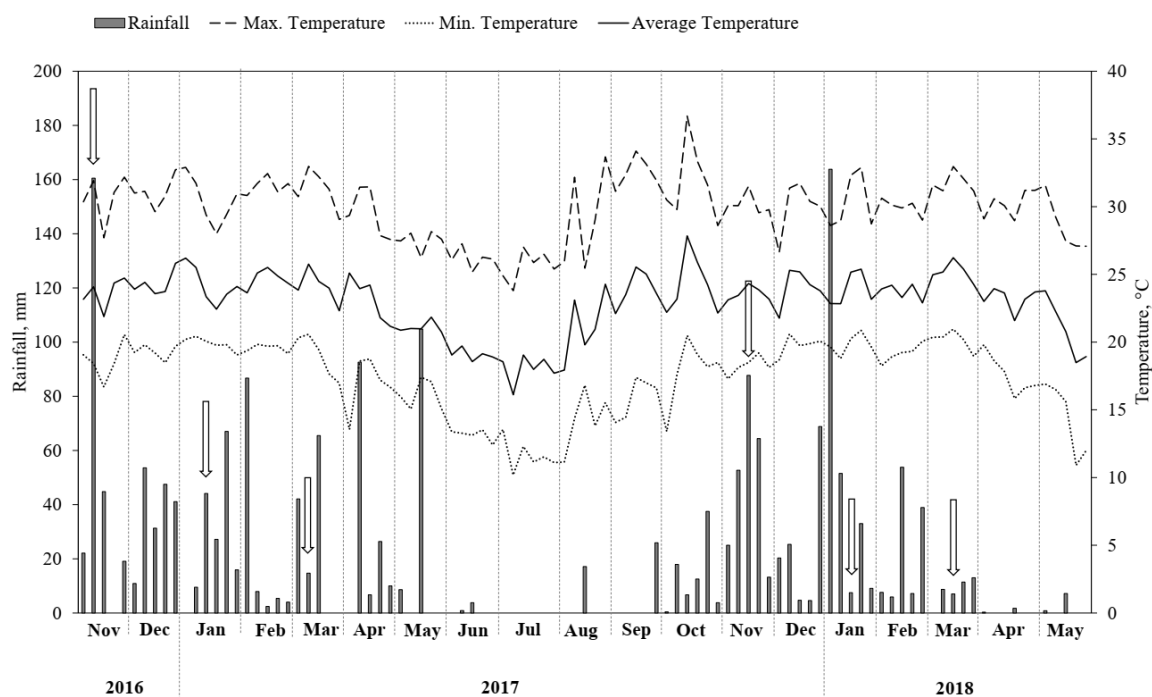


Figure 1. Weekly rainfall and air temperature for the period November 2016 to May 2018 in Jaboticabal, Brazil. Arrows indicate the nitrogen application ($150 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, split in three equal applications) in the fertilized treatment.

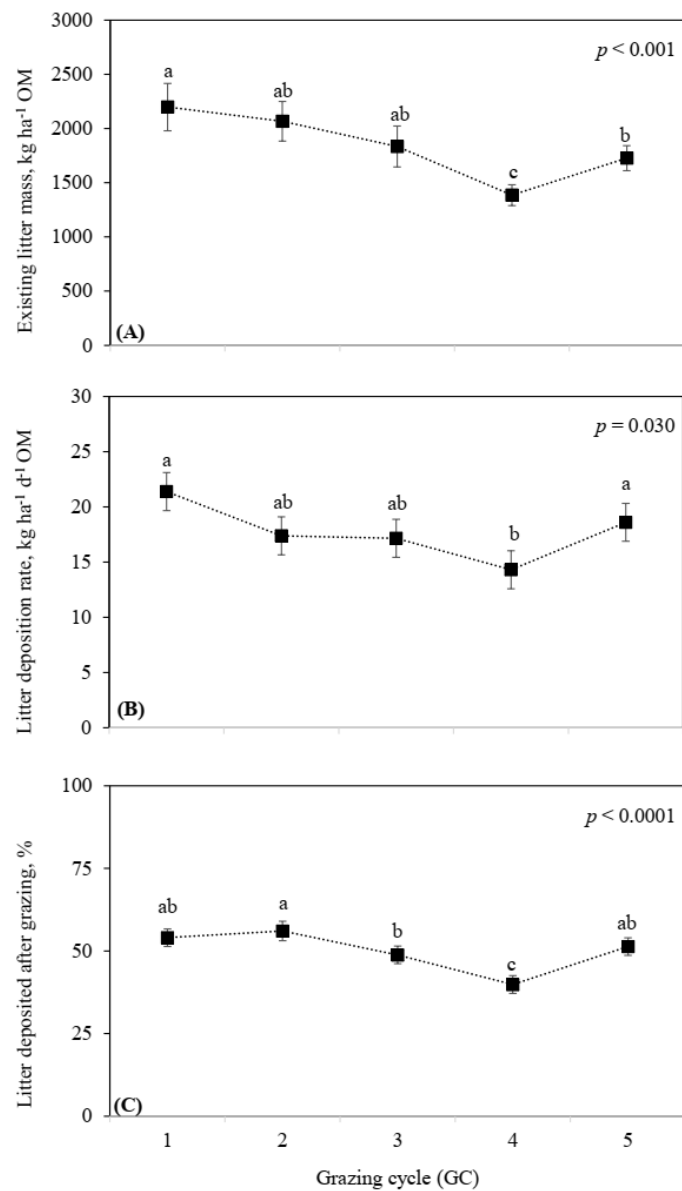


Figure 2. Mean of (A) existing litter mass, (B) litter deposition rate, and (C) litter deposited after grazing for palisadegrass pastures under the three N supply treatments during five grazing cycles (GC). Error bars represent standard error. Same letter indicates no difference according to the PDIFF procedure by LSD test ($p < 0.05$). The response variables are means across three treatments, four replicates, and 2 yr ($n = 24$).

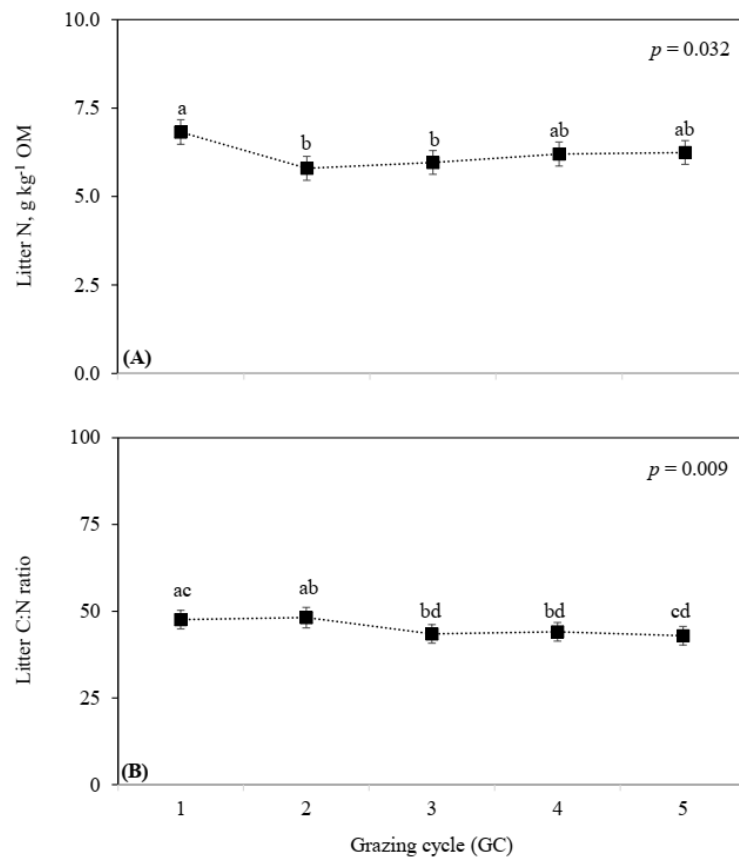


Figure 3. Mean of (A) litter N, and (B) litter C:N ratio for palisadegrass pastures under the three N supply treatments during five grazing cycles (GC). Error bars represent standard error. Same letter indicates no difference according to the PDIFF procedure by LSD test ($p < 0.05$). The response variables are means across three treatments, four replicates, and 2 yr ($n = 24$).

CAPÍTULO 4 – Nitrogen supply and season affect ammonia emissions from dairy cattle excreta and urea fertilizer applied on warm-climate pastures¹

ABSTRACT

Cattle excreta and nitrogen fertilizer deposited on tropical grasslands are important sources of ammonia (NH₃) emission. We conducted three field trials (wet, transition, and dry season) to quantify the NH₃ emissions from urea fertilizer and simulated excretions of heifer urine and dung on warm-climate grasslands in Brazil. Heifer excreta were derived from pastures of palisadegrass [*Urochloa brizantha* (Hochst. ex A. Rich.) R. D. Webster cv. ‘Marandu’] under three forms of N supply [without or with N fertilization (0 or 150 kg N ha⁻¹ yr⁻¹) or mixed with forage peanut (*Arachis pinto* cv ‘Amarillo’)]. Cumulative NH₃-N emission across seasons ranged between 7.6 to 16.6% (mean 11.7%) for urine, 1.4 to 2.9% (mean 2.0%) for dung, and 11.2 to 20.5% (mean 14.8%) for urea. Ammonia loss from urine was significantly greater than from dung for all seasons. Excreta type (urine and dung) differ from urea fertilizer when applied on palisadegrass. There were greater NH₃ emissions from urine in the wetter times of the year. Heifer excreta from N-fertilized pasture had greater NH₃ emission than excreta from the grass-legume mixture and unfertilized palisadegrass. Urea fertilizer applied on palisadegrass presented greater NH₃ emissions in the wet season compared with the dry season but did not differ from the transition season. Our study showed that N-fertilized systems increases N losses as NH₃ emission from excreta, and emissions from urea fertilizer must be included in this system. Heifer excreta and urea fertilizer deposited on warm-climate grasslands increased the NH₃ emission mainly in the wet season.

¹ Este capítulo corresponde ao artigo científico editado nas normas da revista Journal of Environmental Quality.

1. INTRODUCTION

Livestock production in warm-climate environments is based mainly on grassland ecosystems (Ferraz and Felício, 2010). Pastures in Brazil are predominated by the genus *Urochloa* covering an estimated 80 million hectares and approximately 56% of this area is estimated to be occupied by the cultivar ‘Marandu’ of palisadegrass [*Urochloa brizantha* (Hochst. ex A. Rich.) R. D. Webster] (Silva et al., 2016). Large amounts of nitrogen (N) are excreted daily by grazing animals via cattle excreta, which are the primary source of ammonia (NH₃) emission (Phelan et al., 2015). Brazilian herd size is estimated in around 213.5 million heads with approximately 7.7% of dairy cows (IBGE, 2018). Annual losses via this excreta pathway accounts for nearly 26 million tons of N globally (Gerber et al., 2013) and 17 million tons of N globally to synthetic N fertilizer (Xu et al., 2019).

The Intergovernmental Panel on Climate Change (IPCC) suggests a default NH₃ emission factor of 30% (uncertainty range 20 to 50%) for animal excreta and 15% (uncertainty range 3 to 43%) for urea fertilizer for national greenhouse gases (GHG) inventory methodology (IPCC, 2019). These factors do not take into account several differences between environments. Moreover, differences between the proportions of NH₃ lost from different forms of animal excreta and fertilizers are expected.

Nitrogen fertilization can improve forage productivity and forage crude protein (Delevatti et al., 2019; Termonen et al., 2020). However, their use also leads to losses of NH₃, nitrous oxide, nitrate, and ammonium (NH₄⁺) (Zaman et al., 2009). In N-fertilized systems, in addition to NH₃ emission from urine and dung, losses from N fertilizers must be included. Thus, alternative pasture managements can have a large impact on NH₃ losses. One common strategy to reduce the use of fertilizers is the integration of grasses and legumes. Legumes can associate with rhizobia and fix atmospheric-N₂, adding N to the system. As a result, an increase in

herbage productivity and forage nutritive value can be expected (Muir et al., 2014; Phelan et al., 2015). In Brazil, the forage peanut (*Arachis pintoi* Krapov. & W.C. Greg. cv. Amarillo) has a good potential to grow with fast-growing grasses such as *Urochloa* (Andrade et al., 2012; Tamele et al., 2018). Forage peanut in a monoculture can fix between 88 to 244 kg N ha⁻¹ of nitrogen from the atmosphere (Carvalho et al., 2019) and hence it could significantly decrease the use of synthetic N fertilizer.

Although the IPCC considers the same NH₃ emission factor for urine and dung, several studies have showed differences between them, both for tropical and temperate grasslands. Nitrogen losses as NH₃ range from 5 to 30% for urine and from 2 to 12% for dung in tropical grasslands (Lessa et al., 2014; Cardoso et al., 2019b). In temperate grasslands, these values range from 8.7 to 25.5% for urine and from 2.8 to 11.6% for dung (Laubach et al., 2013; Fischer et al., 2016; Burchill et al., 2017). The rate of NH₃ emission from urea, urine, and dung can be influenced by changes in soil and weather conditions, such as air temperature, rainfall, soil pH, nitrification activity, and soil moisture (Sommer and Hutchings, 2001; Zaman et al., 2009; Burchill et al., 2017). The NH₃ emission is generally reduced when the rainfall increases, due to N infiltration, and greater NH₃ losses occurs with dry and warm soil (Saarijärvi et al., 2006; Sanz-Cobena et al., 2011). Those emissions are influenced mainly by the rainfall intensity (Engel et al., 2011; Sanz-Cobena et al., 2011). High soil moisture in the moment of the excreta or urea application can increase the NH₃ losses (Fischer et al., 2016) due to the rapid hydrolysis of urea increasing the soil pH (Zaman et al., 2009). Increasing the air temperature high NH₃ losses occurred through water evaporation (Whitehead and Raistrick, 1991). Seasonal variation in rainfall and temperature, commonly found in warm-climate regions, changes the variables that regulate NH₃ losses.

The diet composition of cattle is another factor that can change urine and dung composition, as well as the total nitrogen excreted (Hristov et al., 2013). The protein content of the diet consumed by cattle has a major influence on the ratio of N excreted in urine and dung (Scholefield et al. 1991). With diets of low protein content, commonly encountered in warm climate grazing systems, the ratio of N excreted in urine to that in dung can often be lower than 1:1 such that N excreted in the dung is the predominant form of excreted N (Macedo et al., 2010; Xavier et al., 2014). Forage peanut-palisadegrass mixtures can increase the crude protein and the higher dry-matter digestibility (Gomes et al., 2018), resulting in better value nutritive than diets with grass only (Muir et al., 2014). In the present study, NH₃ emission across three seasons were quantified from both excreta from heifers and urea fertilizer. The focus was the type of pasture under three forms of N supply that served as forage for the animals from which the excreta came. In this study we assessed (i) how urine and dung affect NH₃ emission; (ii) how excreta from different N supply systems affects the NH₃ emission; and (iii) how the seasons affects those emissions, and the emissions associated with urea fertilizer. We hypothesized that NH₃ emissions from excreta derived from grass-legume system will be lesser than N-fertilized grass.

2. MATERIALS AND METHODS

2.1. Experimental area

The study was conducted on a palisadegrass [*Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster] monoculture pasture (1200 m²), established in 2014 and located at Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil (21°14'05" S e 48°17'09" W; 615 m above sea level). In this area, the mean annual rainfall is 1424 mm and the mean annual air temperature is 22.3 °C. Daily rainfall, air temperature (maximum, average, and minimum), and relative

humidity were obtained from the Agrometeorological Station, Department of Exact Sciences, UNESP, Jaboticabal Campus, located at 700 m away from the experiment site. The soil is an Oxisol (USDA, 2014). Soil properties (0-20 cm depth) has a bulk density of 1.44 g cm^{-3} , contains 340 g kg^{-1} sand, 140 g kg^{-1} silt and 520 g kg^{-1} clay (Claessen, 1997), has a pH (CaCl_2) 5.3, organic matter 32.4 g kg^{-1} , cation exchange capacity $74.8 \text{ mmolc dm}^{-3}$, P (ion-exchange resin extraction method) 10.9 mg dm^{-3} , Mehlich-1 extractable Ca $28.3 \text{ mmolc dm}^{-3}$; Mehlich-1 extractable Mg $9.7 \text{ mmolc dm}^{-3}$, Mehlich-1 extractable K $4.2 \text{ mmolc dm}^{-3}$, base saturation 561 g kg^{-1} , respectively (van Raij et al., 2001).

2.2. Experimental design

The experimental design was a randomized complete block, with five replicates (see Supplemental Figure S1). Treatments were distributed in a factorial combination of two factors: i) type of excreta (urine or dung); ii) the type of pasture under three forms of N supply that served as food for the animals from which the excreta came, i.e. unfertilized palisadegrass (0 kg N ha^{-1}), or palisadegrass + urea (150 kg N ha^{-1} , split in three equal applications), or palisadegrass + legume (*Arachis pinto* Krapov. & W.C. Greg. cv. ‘Amarillo’). Urea fertilizer (50 kg N ha^{-1}) was applied as an additional treatment. A control treatment receiving no type of excreta or urea fertilizer was included to assess the emissions from the air + soil + palisadegrass (background emissions). These treatments were applied on palisadegrass monoculture pasture protected from animal access before the treatments application to avoid any influence from urination or defecation in the area where the excreta were applied. Urine, dung, and urea were applied simulating three rainfall pattern common in the studied area (wet, transition and dry period). The terminology chosen for each season was defined according to the rainfall pattern during the sampling (Fig. 1). The evaluation was carried out from pastures seeded in 2014.

Therefore, the evaluation of ammonia emission on heifers' excreta was carried out three and four years after the pasture establishment.

2.3. Excreta collection

After seven days of animal adaptation to the diet, i.e., animals adaptation to the palisadegrass pastures under three N supply (0, or 150 kg N ha⁻¹ yr⁻¹, or legume), fresh excreta (urine and dung) were collected during the next five days of occupation from five animals in each treatment, in all seasons. Excreta collection started at 6 am in the field. The dung was collected immediately following defecation. The urine was collected immediately after urination, with the help of a bucket. Urine and dung were stored in plastic containers at -5 ± 2 °C until application to avoid risk of NH₃ emission (Chadwick et al., 2018). Before urine and dung application on the area of palisadegrass, total urine from five animals for five days was homogenized by treatment (N supply) and the same procedure was performed for the dung. This homogenization was made to reduce the risk of any individual characteristics of the animals on the excreta that can mask the desired effect of diet and rainfall period on NH₃ emissions.

2.4. Treatment application

Experiments were carried out on a palisadegrass monoculture pasture adjacent to the treatments of grazed palisadegrass pasture. The area had not been treated with any nitrogen (urea fertilizer or animal excreta) during previous two years. To simulate the rainfall pattern urine, dung, and urea treatments were applied on 3-24 May 2017, 4-25 April 2018, and 8-29 June 2018 that represented the wet, transition, and dry season, respectively. Each plot measured 0.4×0.6 m (0.24 m²), totalizing 40 plots per seasonal applications. The dung patches were

simulated by applying 1.6 kg of fresh dung based on the estimates of Braz et al. (2003) and Whitehead (1995), in a circular frame of 19-cm-diameter. One liter (1 L) of urine was applied over an area of approximately 24-cm-diameter to represent an area typically covered by a urination event, a bottle was used to simulate the animal urination. The urine volume was based on the estimates of Braz et al. (2003) and Whitehead (1995). Urea fertilizer was applied per plot by hand at a rate of 50 kg N ha⁻¹ (2.67 g plot⁻¹). A new plot was used for each of the three seasonal applications.

2.5. Ammonia emission measurement (NH₃)

Ammonia emission was evaluated using a semi-open chamber. The methodological description and validation of these chambers using ¹⁵N technique were reported in the studies of Araujo et al. (2009) in Brazil and Jantalia et al. (2012) in USA. Briefly, semi-open chamber (2-L PET bottles; 10-cm diameter) with an acid-soaked foam strip (3-mm thick, 2.5-cm wide, and 25-cm long) was used to capture the NH₃. The chamber was placed on the plot immediately after excreta (urine or dung) or urea fertilizer application. Foam strips were collected and replaced with new strips 1, 3, 5, 9, 14 and 21 days after application of treatments (DAA). Quantification of the NH₄⁺ captured with the foam strips was performed by steam distillation and titration following the method described by Morais et al. (2013).

The ammonia emission rates for each treatment was calculated using total cumulative NH₃ emission following Eq. (1)

$$\text{Ammonia emission, \%} = \frac{\text{NH}_3(\text{treatment}) - \text{NH}_3(\text{soil background})}{\text{N}_{(\text{applied})}} \quad (1)$$

where NH₃(Treatment) is total cumulative NH₃ emission (% total N applied lost as NH₃) for the dung, urine or urea fertilizer treatment, NH₃(Soil background) is total cumulative NH₃ emission (% total N applied lost as NH₃) for the air + soil + palisadegrass without N addition, and N_(applied)

is N application in the area covered by the chamber (kg N ha^{-1}). The total amount of NH_3 emitted was corrected for the affected area by each excreta type, and the calibration factor (1.74) as determined by Araujo et al. (2009) in Brazil and validated by Jantalia et al. (2012) in USA.

The NH_3 flux ($\text{kg ha}^{-1} \text{d}^{-1}$) for each treatment was calculated using cumulative NH_3 emission following Eq. (2)

$$\text{NH}_3 \text{ flux} = \% \text{NH}_3 (\text{treatment}) \times N_{(\text{applied ha}^{-1})} \quad (2)$$

where $\% \text{NH}_3(\text{Treatment})$ is cumulative NH_3 emission ($\%$ total N applied lost as NH_3 by day) for the dung, urine or urea fertilizer treatment and $N_{(\text{applied})}$ is N applied in each plot extrapolated to kg N ha^{-1} .

2.6. Dung and urine analysis

On each day of application, subsamples from the dung and urine to be applied were taken and analyzed for total nitrogen content (Table 1) with LECO model FP-528 instrument (LECO Corporation, St. Joseph, MI). Fecal samples were ground using a Wiley mill (Marconi, Ma680) to pass through a 1-mm stainless steel screen and analyzed for dry matter concentration (DM) according to AOAC (1995; DM, method N. 934.01). After samples were roller milled for 16 h (Arnold and Schepers 2004) and the abundance of ^{13}C ($\%$, Table 1) were analyzed using a mass spectrometer (model Delta V Advantage, Thermo Scientific) coupled to an elemental analyzer (model Flash 2000, Thermo Scientific). The proportion of legume in the dung was analyzed using the ^{13}C abundance technique, and it was calculated by using the equation of Coates et al. (1987), Eq. (3):

$$\%C_{\text{legume}} = \frac{100 \times (\delta^{13}\text{C}_{\text{mixed}} - \delta^{13}\text{C}_{\text{grass}})}{(\delta^{13}\text{C}_{\text{legume}} - \delta^{13}\text{C}_{\text{grass}})} \quad (3)$$

where $\delta^{13}\text{C}_{\text{mixed}}$ is the ^{13}C abundance in the dung from palisadegrass-forage peanut mixed pasture, $\delta^{13}\text{C}_{\text{grass}}$ is the palisadegrass pasture, and $\delta^{13}\text{C}_{\text{legume}}$ is the forage peanut pasture (Cantarutti et al., 2002).

2.7. Statistical analyses

Data were analyzed as a randomized block design using the GLIMMIX procedure of the SAS statistical package (SAS Inst. Inc., Cary, NC, USA). Data were checked for normality by assessing residual normality and variance. Total cumulative NH_3 emissions required a natural log transformation. For the analysis of variance, excreta type, N supply, season, and their interactions were considered as fixed effects. Block was the random effect. Degrees of freedom were calculated using the Kenward-Roger 2 option. The LSMEANS were compared using the PDIFF procedure adjusted for LSD test. Differences were considered significant at $p \leq 0.05$ and trends at $p < 0.10$. Preplanned contrasts were used to compare average responses of excreta type (urine and dung) with fertilizer (urea).

3. RESULTS

3.1. Weather conditions

The average air temperatures during the measurement periods were 21.0, 22.9 and 21.3°C and the average relative air humidity were 76.0, 62.3 and 59.6% during the wet, transition, and dry seasons, respectively (Fig. 1dhl). Total rainfall varied greatly between experimental periods. During the wet season, the total rainfall was 114 mm, which was greater than 13, and 0 mm for transition, and dry seasons, respectively. In the wet season, a light rainfall (8.6 mm) occurred at the 2nd day after application of the treatments, while between the 15th and the 18th days the cumulative rainfall was 104.8 mm (Fig. 1d). During the transition season, there was a

light rainfall (11.0 mm) four days before the treatment application, and only one rainfall event (1.7 mm) at the 13th day after treatment application (Fig. 1h). For the dry season, there was no rainfall (Fig. 1l), which explains the low relative air humidity.

3.2. *Effect of excreta type and seasons on NH₃ emission*

There was an excreta type $\times$ season interaction affecting cumulative NH₃ emission ($p < 0.0001$, Fig. 2) i.e., rainfall patterns affected each excreta differently. Ammonia emissions from urine were higher than from dung in all seasons. While for urine, the dry season was the period with lowest emissions, for dung, the transition period led to greater losses than wet or dry periods. When urine was applied on the palisadegrass in the wet season, the greatest NH₃ losses occurred within the first 72 hours of evaluation (Fig. 1a) with 45% of the total cumulative NH₃ emitted over the 21-day evaluation period (Fig. 1b; see Supplemental Table S1). In the transition and dry season, the NH₃ emission peaks occurred within the first 24 hours of evaluation (Fig. 1ei), when expressed as a proportion of the total cumulative NH₃ emitted in the 21-day of evaluation, ranged from 23% in the dry season and 40% in the transition season (Fig. 1fj; see Supplemental Table S1). Greater NH₃ emission peaks for dung occurred within the first 72 hours of evaluation (Fig. 1aei) in all seasons, ranging from 50 to 66% of the total cumulative NH₃ emitted during the 21-day of evaluation (Fig. 1bfj; see Supplemental Table S1). For both excreta types, the NH₃ emission increased to 80% of all emitted within 9 DAA (see Supplemental Table S1). Approximately 95% of total NH₃ emitted during 21-day of evaluation was lost in 14 DAA.

3.3. *Effect of N supply and seasons on NH₃ emission*

There was a N supply $\times$ season interaction affecting on cumulative NH₃ emission (Fig. 3; $p = 0.0025$). In the wet season, the cumulative NH₃ emission of the excreta from animals grazing in unfertilized palisadegrass and palisadegrass + forage peanut was significantly lower than that of the excreta from the palisadegrass + urea. There was no difference in the cumulative NH₃ emission of the excreta from animals grazing palisadegrass with three forms of N supply in the transition season. Cumulative NH₃ emission of the excreta from palisadegrass + forage peanut in the dry season was lower than excreta from unfertilized palisadegrass and palisadegrass + urea. Regarding the effect of seasons within excreta from different N supply, no difference was found on cumulative NH₃ emissions between seasons for excreta from unfertilized palisadegrass. There were greater cumulative NH₃ emissions in the wet and transition season than in the dry season for excreta from palisadegrass + urea, and the greatest cumulative NH₃ emission was in the transition season for excreta from palisadegrass + forage peanut.

3.4. *NH₃ emission from urea*

Urea fertilizer had a trend on cumulative NH₃ emission over the season (Fig. 2; $p = 0.0815$). Ammonia losses were significantly higher in the wet season than in the dry season, the transition season showing intermediate values. When urea was applied on the palisadegrass in the wet season, the NH₃ emission peaks occurred on the 5th DAA (Fig. 1a), with losses of 61% of the total NH₃ emitted (Fig. 1b) and up to 83% in the 9th DAA. The pattern of urea fertilizer in the transition and the dry season were different from the wet season (Fig. 1ei). Ammonia emission peaks were not so evident as for excreta (urine and dung), but they occurred in the first 72 hours of evaluation. When expressed as a proportion of the total NH₃ emitted in the 21-

day of evaluation, ranged from 20% in the transition and dry season (Fig. 1fj). Two weeks after application of treatments, NH_3 losses increased to 98, 76, and 92% of the total cumulative NH_3 emitted for the wet, transition and dry season, respectively. Greater cumulative NH_3 emission was found to fertilizer when compared to excreta type ($p < 0.0001$; Fig.2).

4. DISCUSSION

4.1. *Urine vs. dung on NH_3 emission*

The fraction of N lost as NH_3 from urine was almost 10, 4, and 5 times greater than that from dung in the wet, transition, and dry season, respectively. Other researchers have observed differences between urine and dung NH_3 emission from tropical grasslands (Lessa et al., 2014; Cardoso et al., 2019b) and temperate grasslands (Laubach et al., 2013; Fischer et al., 2016; Nichols et al., 2018). Differences occur due to the different physical and chemical composition of dung and urine. The N present occurs in different forms, such as urea for urine and organic-N in dung. The N in dung is bound in undigested dietary proteins and bacterial cells such as residues of secreted enzymes, sloughed intestinal cells and of the gastro-intestinal biota (Van Vuuren and Meijs, 1987; Oenema et al., 1997). Urea (source of NH_4^+) can vary between 43 to 70% in the urine (Cardoso et al., 2019a) and the proportion is influenced by the composition of the diet. Nitrogen present in the dung is to a large extent recalcitrant (Lessa et al., 2014) thus, only very slow release of NH_4^+ occurs via mineralization. Another explanation for the lower NH_3 emission in dung as compared to urine is the formation of a superficial crust on the dung. This crust can act as a physical barrier to the wind preventing the NH_3 emission. Furthermore, NH_3 tends to diffuse between fecal matter making soil surface contact, in which it will be converted into NH_4^+ , making NH_3 emission even more difficult (Mulvaney et al.,

2008). Similar results were found by other authors (Petersen et al., 1998; Lessa et al. 2014; Fischer et al., 2016; Cardoso et al., 2019b).

4.2. *Urine and dung across seasons on NH₃ emission*

Cumulative NH₃ emission for urine applied on palisadegrass ranged between 7.6 to 16.6% (mean 11.7%) of urine-N across the three seasons and was similar to the range of emission (5 – 30%) reported by others for urine in tropical grasslands (Table 2). The fraction of N lost as NH₃ in the wet seasons was almost twice that lost in the dry season (Fig. 2). This result does not corroborate with recent work in tropical grasslands. In conditions similar to those in this study, the NH₃ emission ranged from 7.6 to 20.9% of N losses from urine in the dry season and 5.0 to 7.6% in the wet season (Cardoso et al., 2019b). The authors suggested that the presence of water decreases NH₃ emission by the N incorporation into the soil. Probably in our study the wet season was misleading, there was a light rainfall (8.6 mm) at the beginning, while most of the rain occurred at the end. Therefore, during the critical period for NH₃ losses, the conditions were not wet at all. This light rainfall could have stimulated the emission, but if the 100 mm had occurred at the beginning, NH₃ losses would have been much lower due to the N infiltration or runoff. In the transition season, a small rainfall on the 4th day before treatment application, may have affected the initial soil moisture retarding urine infiltration (Fischer et al., 2016). Therefore, the greatest NH₃ emission peak (~5 kg ha⁻¹ d⁻¹) was on the 1st DAA. There was a prolonged period of drought in the dry season (Fig. 1l), with no rainfall occurring before or after urine application which restricted urea hydrolysis, resulting in the lowest NH₃ emission peaks (~2 kg ha⁻¹ d⁻¹). Lessa et al. (2014) reported that 80% of NH₃ emission occurred within the first 2 days after urine application in the wet period. Burchill et al. (2017) reported ammonia losses from urine were rapid, with 33 to 44% of the total NH₃ emission occurring

within the first 24 h on both application dates (May and June) and up to 80% within three to four days.

Cumulative NH_3 emission for dung applied on palisadegrass ranged between 1.4 to 2.9% (mean 2.0%) across the three seasons. In our study, losses from dung were much smaller than in previous research (Table 2). The fraction of N lost as NH_3 from dung in the transition season was almost twice that in the wet and dry season. Cardoso et al. (2019b) did not find NH_3 emission to be different between seasons (7.2% in the wet season and 6.0% in the dry season), while Lessa et al. (2014) found greater NH_3 emission in the dry season (4.3%) compared to rainy season (2.5%). However, in our present study we found a greater NH_3 emission in the transition season, with reduced frequency of rainfall, and greater air temperature than in the wet and dry season (Fig. 1dhl). Ammonia emission is typically greatest at high temperatures (Holtan-Hartwig and Bockman, 1994). Furthermore, the dung in that season presented lower dry matter concentration ($14.9 \pm 0.7\%$ DM) compared to wet and dry season ($19.2 \pm 1.0\%$ and $17.0 \pm 0.4\%$ DM, respectively). This greater moisture may have delayed the formation of a surface crust on the dung patches as explained previously. Besides that, the rainfall (11.3 mm) that happened four days before the application may have maintained soil moisture.

4.3. Combined effect of N supply and seasons on NH_3 emission

Greater NH_3 emission was found from excreta from heifers fed with palisadegrass + urea than heifers fed with palisadegrass + forage peanut. The fertilization or the legume inclusion may change the forage N concentration, which is also affected by weather conditions. These factors may influence the N losses from heifers' excreta by the changes in the pasture chemical composition consumed. During the urine and dung collections, the pasture crude protein was 8.4, 11.4, and 9.1% (mean 9.6%) in the wet season, 8.3, 11.5, and 8.1% (mean 9.3%) in the

transition season, and 7.3, 9.7, and 7.2% (mean 8.1%) in the dry season, for the unfertilized palisadegrass, palisadegrass + urea, and palisadegrass + forage peanut, respectively. Pasture N fertilization may have increased the A protein fraction that is highly degradable in the rumen, which may have provided greater urea excretion from urine or organic N compounds from dung. Smits et al. (1995) found a reduction in NH_3 emission rate by 40% when dairy cows received diets with a lower rumen-degradable protein comparing to diets with greater amount of high-degradable proteins. The excessive NH_3 in the rumen is absorbed through the rumen wall and converted to urea in the liver, which is loss in the urine (Lewis, 1957; Hristov et al., 2011). The urea excretion in the urine was found to increase linearly with the level of N fertilization of palisadegrass (Aguilar et al., 2016). Greater NH_3 emissions occur in urine with greater urea amount. Another possible explanation for the greater NH_3 emission is the lower dry matter concentration for dung from the palisadegrass + urea (17.1 and 16.5% DM) compared to palisadegrass + forage peanut (21.2 and 18.0% DM). This greater moisture may have delayed the formation of a surface crust on the dung patches as explained previously.

Our study suggests that palisadegrass + forage peanut could be an option to reduce NH_3 losses in the system. Legume inclusion is a strategy to add N via biological dinitrogen fixation (BNF) which reduces the need to apply synthetic fertilizers, such urea (see Supplemental Table S2). This is a key factor in the increased productivity of the system and mitigation of NH_3 emissions. However, our study showed that NH_3 emission was also influenced by season, indicating that the effect of heifers' excreta fed palisadegrass with different N supply strategies on NH_3 emission is season dependent. We are highlighting the need to consider additional information, such as soil parameters, legume species and different weather regions. Ammonia mitigation from excreta has been attributed to the presence of secondary compounds in the legume, such tannins and saponins (Hess et al., 2006). However, the presence of these

compounds was not evaluated in this study. In New Zealand, Laubach et al. (2013) reported NH_3 emission of 12 and 26% (dung and urine, respectively) for cattle fed with harvested forages, mainly perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). These results are considerably higher than those found in the current study. The difference in the weather, pasture species, and the ammonia measurement methodology probably explains the differences in the results. Another explanation is the season, and the greater N concentration in the diet may influence water intake and lead to an N dilution in the urine (Selbie et al., 2015).

4.4. Effect of urea and season on NH_3 emission

Our results were somewhat lower than those reported from a study on tropical grasslands of 1 – 53% (mean 19%; Table 2) and temperate grasslands 8 – 53% (Forrestal et al., 2016; Burchill et al., 2017). The measurement method type in some studies may overestimate the NH_3 emission. Ryden and Lockyer (1985) and Forrestal et al. (2016) found that wind tunnels can overestimate NH_3 emission. In our study, we utilized a semi-open chamber developed by Araujo et al. (2009) in Brazil and Jantalia et al. (2012) in USA. Cardoso et al. (2019b) used the same semi-open chamber used for us, and they found similar NH_3 emission from urea (11 – 23%).

Urea fertilizer showed a variation in NH_3 emissions similar to that for urine among seasons (Fig. 2). The N fraction lost as NH_3 from urea in the wet season was almost twice that in the dry season. A small quantity of rain that fell on the 2nd DAA the urea application in the wet seasons probably stimulated the NH_3 emission due to the urea hydrolysis (Fig. 1dh). Soil moisture and air humidity are absorbed by the urea granules resulting in hydrolysis and the NH_3 loss (Forrestal et al., 2016). Engel et al. (2011) found greater NH_3 emission occurring in an initial wet soil followed by a drying period than in an initial dry soil followed by a significant

rainfall event. A low water irrigation/rainfall volume (<7 mm) after the urea application increases NH_3 emission by hydrolysis, whereas irrigation/rainfall with higher intensities (>7 mm) tend to reduce the emission caused by the NH_3 infiltration in the soil (Engel et al., 2011; Sanz-Cobena et al., 2011). After the urea hydrolysis in the soil, the NH_4^+ ions would be immobilized on cation exchange sites (Sanz-Cobena et al., 2011). Probably in our study the wet season was atypical, there was a light rainfall (8.6 mm) at the beginning, while most of the rain occurred at the end. Therefore, during the critical period for NH_3 losses, the conditions were not wet at all. This light rainfall could have stimulated the emission, but if the 100 mm had occurred at the beginning, NH_3 losses would have been (likely) much lower due to the incorporation of the urea. Rainy seasons or wet areas do not lead necessarily to higher NH_3 losses than dry areas/seasons. It depends mostly on the amount of rain or irrigation water in the first days after N application in the soil (Sanz-Cobena et al., 2011). During the dry season, the NH_3 emission was low in the first days after the application of urea on palisadegrass. The absence of rainfall, and lower temperature (21.3°C) and relative air humidity (59.6%) in the dry season may have resulted in a longer period than 21 days on NH_3 emission (Fig. 1ij). Fischer et al. (2016) found greater NH_3 losses due to a prolonged emission period caused by dry conditions.

5. CONCLUSION

Urine and dung deposited on warm-climate grasslands are indeed important sources of NH_3 emission. Chemical and physical compositions between excreta provide greater emissions for urine than for dung across all seasons. This study has provided information that palisadegrass + forage peanut pastures may be a possible alternative to reduce N losses by NH_3 emission in the system, directly (lower emissions from the excreta) and indirectly (lower emissions if the

previous pasture is not fertilized and through BNF). Palisadegrass + urea pastures resulted in the greatest NH_3 emission from excreta, and emissions from urea fertilizer must be included in this system. Changing the heifers' diet is a strategy to mitigate the NH_3 emission from excreta and indirectly from the pasture system. Overall, greater NH_3 emission occurred in the wet season than in dry season. Ammonia emission from urea was like the default value estimated by the Intergovernmental Panel on Climate Change (IPCC, 2019). However, urine and dung NH_3 emission was found lower than the default value, indicating that a single emission factor for excreta may not be appropriate. Aggregating emission data of different Brazilian weather regions and different animal categories will be necessary to provide country-specific emissions data to be used in agriculture inventory calculations.

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Tables

Table 1. Amount of N concentration in the heifer urine, dung, and urea fertilizer applied on palisadegrass as treatment, and proportion of forage peanut in the dung in the mixed treatment according to the season experimental.

	Wet	Transition	Dry
	Urine (g N L ⁻¹ plot ⁻¹) [§]		
Unfertilized palisadegrass	3.14 ± 0.22 ^a	2.04 ± 0.17	2.16 ± 0.07
Palisadegrass + urea	4.47 ± 0.40	3.04 ± 0.07	2.48 ± 0.17
Palisadegrass + forage peanut	4.55 ± 0.09	2.39 ± 0.04	2.76 ± 0.07
Means	4.06 ± 0.23	2.49 ± 0.13	2.47 ± 0.10
	Dung (g N plot ⁻¹)		
Unfertilized palisadegrass	3.43 ± 0.20	3.78 ± 0.01	3.89 ± 0.05
Palisadegrass + urea	3.13 ± 0.04	3.56 ± 0.04	4.22 ± 0.03
Palisadegrass + forage peanut	3.40 ± 0.06	3.85 ± 0.08	3.80 ± 0.04
Means	3.32 ± 0.09	3.73 ± 0.06	3.97 ± 0.08
	Fertilizer (g N plot ⁻¹)		
Urea	1.20	1.20	1.20
	Forage peanut		
δ ¹³ C (‰)	-13.3	-14.6	-13.9
Forage peanut in dung (%) [†]	15.3	15.0	11.2

[§]One liter or 1.6 kg of fresh dung or 2.67 g of urea were applied per plot according to the treatment.

[†]The proportion of legume in the dung in the mixed treatment was determined by ¹³C abundance. The ¹³C abundance of forage peanut was -26.3, -28.3 and -28.3‰ for wet, transition, and dry season, respectively.

^aStandard error.

Table 2. Summary of the literature reported on ammonia emission factors (% N loss as NH_3) for cattle excreta (dung and urine) and urea fertilizer applied on tropical grasslands.

N source	Method [§]	% N loss as NH_3	References	Country
Urine	SCC	6–11	Majumdar et al. (2006)	India
Urine	SOC	17–30	Lessa et al. (2014)	Brazil
Urine	SOC	5–21	Cardoso et al. (2019b)	Brazil
Urine	SOC	7–17	This study	Brazil
Means value		14 (5–30)		
Dung	SOC	2–4	Lessa et al. (2014)	Brazil
Dung	SOC	2–12	Cardoso et al. (2019b)	Brazil
Dung	SOC	1–3	This study	Brazil
Means value		4 (1–12)		
Urea	CC	3–25	Ruess and McNaughton (1988)	Tanzania
Urea	MB	20	Prasertsak et al. (2001)	Australia
Urea	SOC	44–53	Morais et al. (2013)	Brazil
Urea	MBM	2–30	Suter et al. (2013)	Australia
Ammonium-N nitrate	MB	1–9	Suter et al. (2013)	Australia
Urea	HFT	27	Schwenke et al. (2014)	Australia
Urea	SOC	11–23	Cardoso et al. (2019b)	Brazil
Urea	SOC	11–21	This study	Brazil
Means value		20 (1–53)		

[§]CC – Closed chambers; SCC – Static closed chambers; SOC – Semi-open chambers; HFT – Horizontal flux technique; MB – Mass balance; MBM – Mass balance micrometeorological method.

Figures

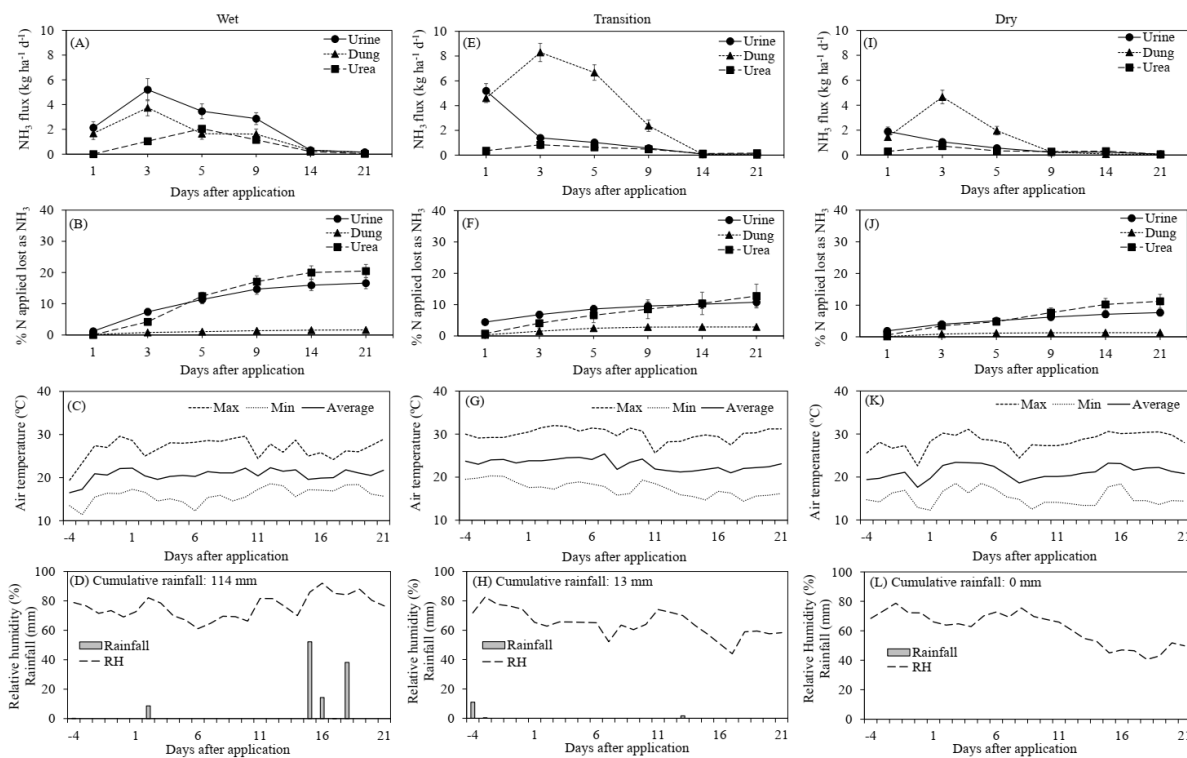


Fig. 1. Daily NH_3 flux ($\text{kg ha}^{-1} \text{d}^{-1}$) and cumulative NH_3 emission (% N applied lost as NH_3) for urine, dung, and urea during wet, transition, and dry season. Error bars refer to the standard error of the mean. Data are means across five replicates ($n = 5$). Daily rainfall, air temperature (minimum, average and maximum), and air relative humidity (RH) for each experimental period. Data from Agrometeorological Station, Department of Exact Sciences, UNESP, Jaboticabal Campus, located at 700 m away from the experiment site.

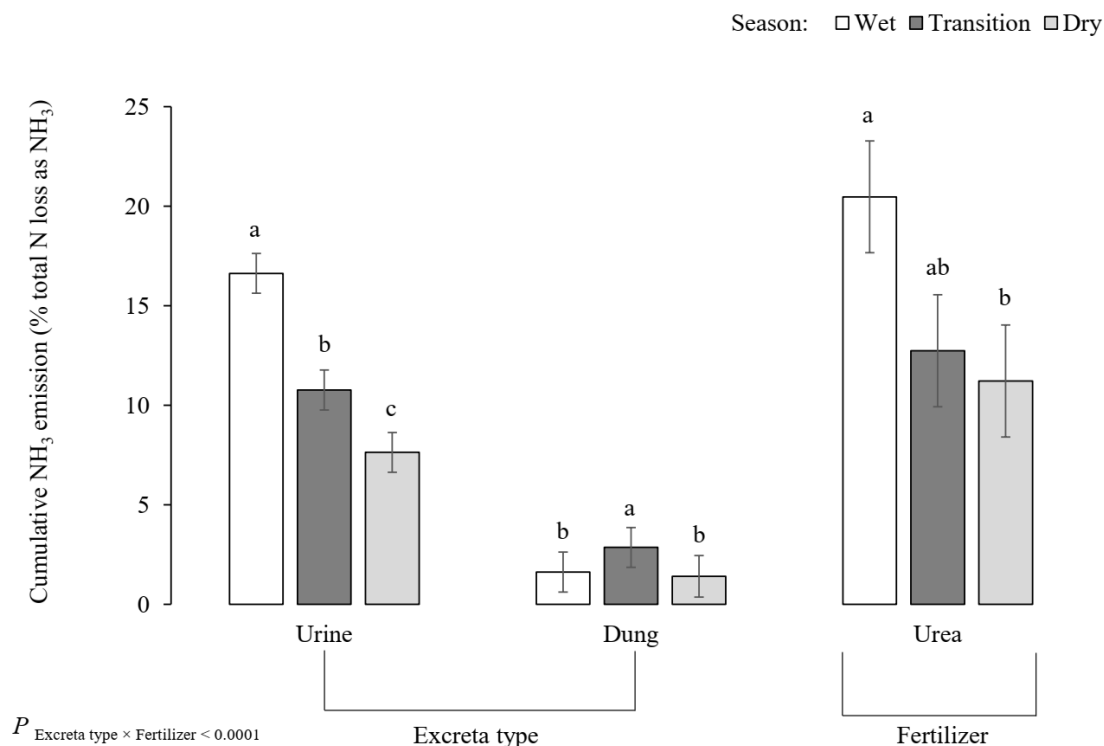


Fig. 2. Cumulative NH₃ emission was affected by excreta type $\times$ season ($p < 0.0001$, SE = 1.0), and fertilizer $\times$ season ($p = 0.0815$, SE = 2.8). Error bars refer to the standard error of the mean (SE). Data are means across five replicates ($n = 5$). Different lowercase letter within season are different according to the PDIFF procedure adjusted by LSD test ($p \leq 0.05$). $P_{\text{Excreta type} \times \text{Fertilizer}}$, P -value for comparison of the average of the two excreta treatments (urine and dung) vs. the average of the fertilizer (urea); § Season ($p < 0.0001$), excreta type ($p < 0.0001$), excreta type $\times$ N supply ($p = 0.3035$), season $\times$ excreta type $\times$ N supply ($p = 0.0676$).

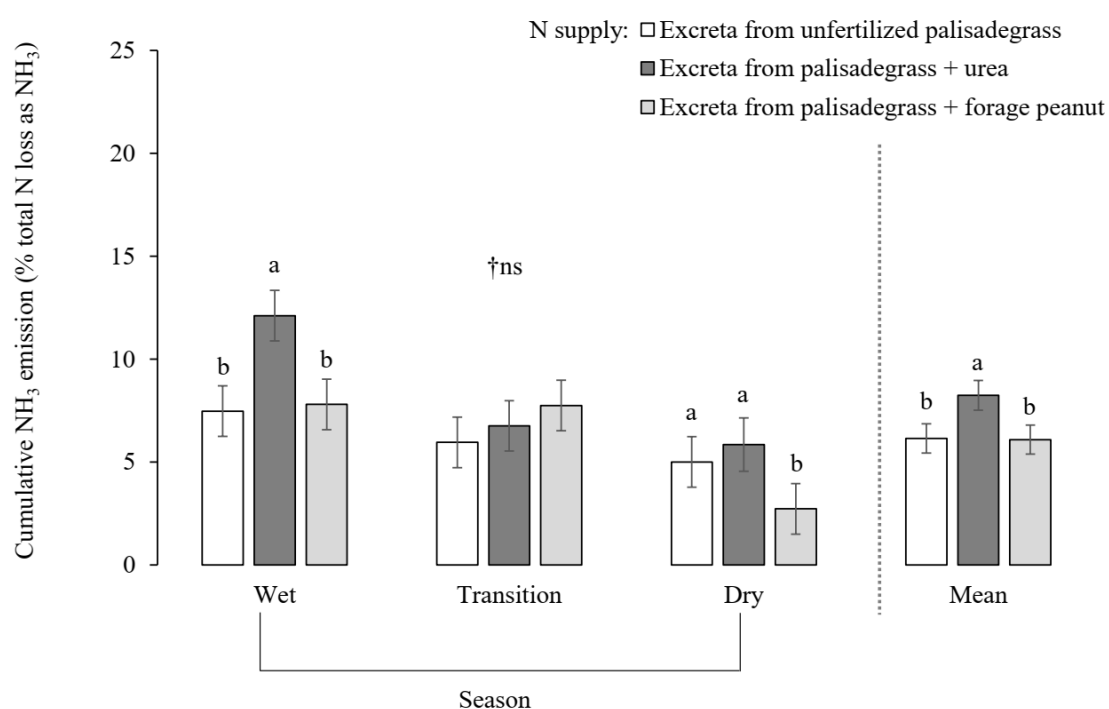
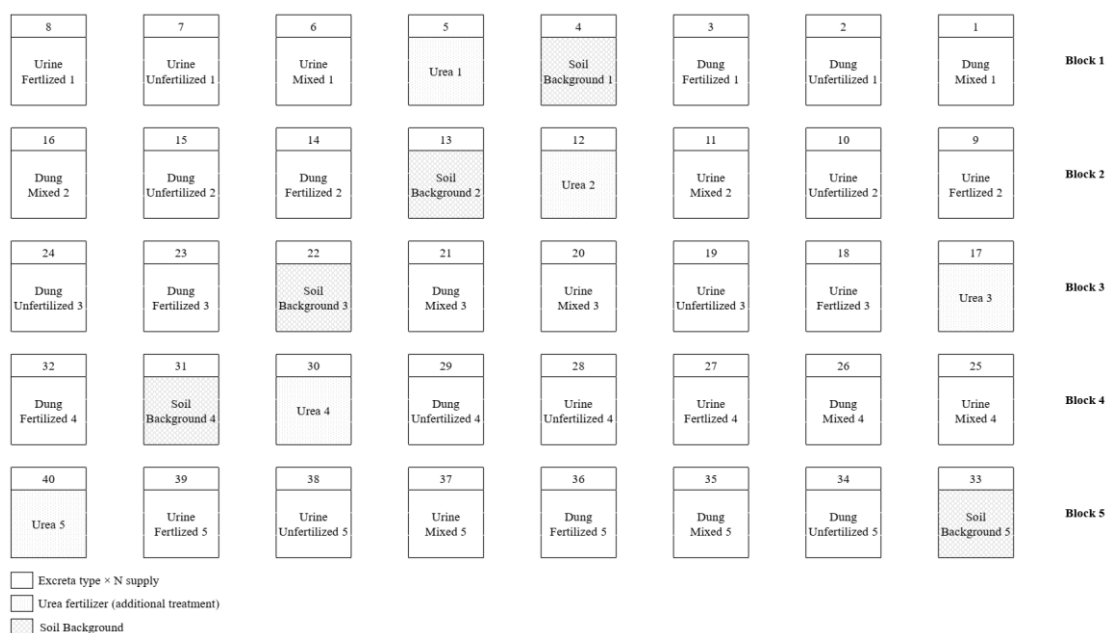


Fig. 3. Cumulative NH_3 emission from excreta (urine and dung) was affected by N supply $\times$ season ($p = 0.0025$, $\text{SE} = 1.2$), and N supply ($p < 0.0001$). Error bars refer to the standard error of the mean (SE). Data are means across five replicates ($n = 5$). Different lowercase letter within season are different according to the PDIFF procedure adjusted by LSD test ($p \leq 0.05$).

Supplementary



Supplemental Figure S1. Experimental area with five replicates in a randomized block design (40 chambers). Two excreta types (urine or dung) from palisadegrass pastures under three N supply (unfertilized or fertilized or mixed), and an additional treatment (urea fertilizer). Soil background to quantify the emission from the air + soil + palisadegrass. These treatments were applied in three seasons (wet, transition, and dry).

Supplemental Table S1. Cumulative percentage of total NH₃ emitted during 21-day of evaluation

DAA [†]	1	3	5	9	14	21
Wet season						
Dung	10	50	65	79	93	100
Urine	8	45	68	88	96	100
Urea	0	21	61	83	98	100
Transition season						
Dung	12	53	86	98	99	100
Urine	40	60	76	84	91	100
Urea	6	30	48	62	76	100
Dry season						
Dung	10	66	88	95	98	100
Urine	23	49	65	80	93	100
Urea	7	30	43	69	92	100

[†] DAA = days after application of treatments

Supplemental Table S2. Ammonia emission of each grazing system from which the treatments came

Grazing system	Urine	Dung	Urea	Biological N Fixation
	———% N loss as NH ₃ §———			kg N ha ⁻¹
Unfertilized palisadegrass	10.7 ± 1.2	1.6 ± 0.2	—	—
Palisadegrass + urea †	13.8 ± 2.0	2.6 ± 0.3	14.8 ± 1.9	—
Palisadegrass + forage peanut ‡	10.6 ± 1.8	1.6 ± 0.3	—	13.5 ± 1.8

† In the palisadegrass + urea, urea was applied 50 kg N ha⁻¹ three times (150 kg N ha⁻¹ yr⁻¹)

‡ In the palisadegrass + forage peanut, the forage peanut contributed with the systems fixing the N atmospheric at 13.5 ± 1.8 kg N ha⁻¹ for 5 months

§ Means among season

CAPÍTULO 5 – Considerações finais

O uso de sistemas que sejam sustentáveis desempenha papel fundamental para manter o equilíbrio no ecossistema das pastagens e reduzir impactos ambientais, causados principalmente pelo uso de adubos nitrogenados sintéticos. A inclusão de leguminosas forrageiras em consórcio com gramíneas são consideradas promissoras na substituição do uso de adubos nitrogenados, como a ureia. No entanto, essa prática ainda é pouco empregada no Brasil. Aspectos relacionados ao lento estabelecimento da leguminosa, baixa disponibilidade de sementes no mercado, bem como pelo alto preço das sementes e a complexidade de instalação desses sistemas são os principais entraves para sua adoção.

Atualmente, no estado de São Paulo, o preço de 1 kg de semente de *A. pinto* cv. 'Amarillo' é de R\$ 249,00 (BRSEEDS, 2019). Para o plantio de 1 hectare em sistema consorciado, são necessários 10 kg de sementes do amendoim forrageiro, resultando em um investimento de R\$ 2490,00 + custos de implantação. Recentemente, a Embrapa-Acre lançou a semente do cultivar BRS Mandobi, por ser de origem brasileira, a implantação de sistemas consorciados com amendoim forrageiro se tornará mais acessível ao produtor rural. O preço atual de 1 kg dessa semente é de R\$ 100,00, reduzindo em 60% o custo de implantação por hectare.

Para sistemas que utilizam a adubação com ureia, no Estado de São Paulo, a tonelada da ureia em janeiro de 2020 foi cotada a R\$ 1562,24 sem contabilizar o custo do frete (Scot consultoria). Para adubações de 150 kg N ha⁻¹ ano⁻¹, são necessário 333 kg de ureia. Na primeira instância, a utilização da ureia é vista como a mais economicamente viável, comparada com os gastos de implantação do consórcio. No entanto, gastos com adubos nitrogenados ocorrem anualmente nos custos de produção do produtor, sendo o preço anualmente afetado pela demanda do mercado. Além disso, questões relacionadas à impactos ambientais ocorrem desde o momento da fabricação do adubo nitrogenado até após a aplicação nos pastos. Em contrapartida, os sistemas consorciados são considerados ecologicamente viável ao ecossistema, e o maior custo de produção será no momento de semeadura até total estabelecimento da leguminosa.

No presente estudo, não foram encontradas diferenças entre sistema consorciado e o sistema de gramíneas em monocultura não adubadas, tanto para os aspectos relacionados à morfologia das forrageiras (CAPÍTULO 2), como aos relacionados à decomposição da serapilheira (CAPÍTULO 3). Em contrapartida, o sistema adubado com 150 kg N ha⁻¹ apresentou maior produção de forragem pré-pastejo e deposição de serapilheira. Provavelmente o menor desempenho do sistema consorciado ocorreu em virtude ao lento estabelecimento do amendoim forrageiro, bem como pela a baixa proporção da leguminosa na composição botânica ($6 \pm 3\%$ de amendoim forrageiro). No entanto, resultados satisfatórios de desempenho animal, produção de forragem e ciclagem de nutrientes são verificados em estudos desenvolvidos no Brasil utilizando o *Arachis pinto* (GOMES et al., 2018; TAMELE et al., 2018; PEREIRA et al., 2020) e na Flórida-EUA utilizando o *Arachis glabrata* (DUBEUX et al., 2017; JARAMILLO et al., 2018; KOHMANN et al., 2019) após o completo estabelecimento da leguminosa no sistema consorciado.

Outro enfoque desta pesquisa foi a avaliação da volatilização de NH₃ nas excretas de novilhas alimentadas com capim-marandu adubados com ureia ou em consórcio com amendoim forrageiro (CAPÍTULO 4). Avaliando os sistemas de forma geral, o consórcio pode ser uma boa alternativa para reduzir as perdas de nitrogênio de forma direta e indireta. A forma direta ocorre pela menor volatilização de NH₃ nas excretas das novilhas. A forma indireta é devido ao não uso da ureia, pois a incorporação do nitrogênio no sistema acontece de forma sustentável, via fixação biológica do N atmosférico. Visto que a ureia pode perder o nitrogênio entre 10 a 21% via volatilização de NH₃. Para os sistemas adubados, a contabilização do nitrogênio emitido para o ambiente via volatilização de NH₃, devem incluir as perdas pela aplicação da ureia no pasto + as perdas nas excretas (fezes e urina) das novilhas.

Mudanças nas condições ambientais (precipitação e temperatura) foram as maiores reguladoras no desenvolvimento da pastagem, bem como nos aspectos de ciclagem de nutrientes e na volatilização de amônia das excretas. Em nosso estudo observamos dificuldade no estabelecimento do amendoim forrageiro em consórcio com capim-marandu, mas notamos a persistência da leguminosa no sistema de um ano para o outro. Alguns pontos como a forma de semeadura nas entre linhas e a baixa pressão de pastejo antes do início do período experimental podem ter afetado

negativamente o desenvolvimento da leguminosa. Dessa forma, a semeadura da leguminosa em faixas possivelmente pode melhorar o desenvolvimento do amendoim forrageiro em consórcio com gramíneas. Embora essa leguminosa tolere sombreamento, uma das respostas morfológicas a esse efeito é o alongamento do colmo de maneira inversa à emissão de estolões. Portanto, o início de pastejo antes de 90% de interceptação luminosa e altura de resíduo abaixo de 15 cm do capim-marandu, principalmente no período de estabelecimento do amendoim forrageiro poderiam trazer benefícios à emissão de estolões.

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