# DINÂMICA DA SERAPILHEIRA E FLUXOS DE GASES DE EFEITO ESTUFA EM PLANTIOS DE EUCALIPTO E VEGETAÇÃO NATIVA DO CERRADO

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TESE DE DOUTORADO EM CIÊNCIAS FLORESTAIS

**PUBLICAÇÃO: PPGEFL.TD-089/2018**
**BRASÍLIA/ DF: MARÇO DE 2018**

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TESE DE DOUTORADO SUBMETIDA AO DEPARTAMENTO DE ENGENHARIA FLORESTAL DA FACULDADE DE TECNOLOGIA DA UNIVERSIDADE DE BRASÍLIA, COMO PARTE DOS REQUISITOS NECESSÁRIOS À OBTENÇÃO DO GRAU DE DOUTORA EM CIÊNCIAS FLORESTAIS.

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**BRASÍLIA/DF, 23 DE MARÇO DE 2018.**

## FICHA CATALOGRÁFICA

Ribeiro, Fabiana Piontekoswski
Dinâmica da serapilheira e fluxos de Gases de Efeito Estufa em plantios de eucalipto e vegetação nativa do Cerrado.

Fabiana Piontekowski Ribeiro, -- 2018

xii 83 p. : 210 x 297 mm

Tese (Doutorado) Universidade de Brasília, Faculdade de Tecnologia, Departamento de Engenharia Florestal. Programa de Pós-graduação em Ciências Florestais.

1. Qualidade da serapilheira. 2. Híbridos de *Eucalyptus urophylla* x *Eucalyptus grandi.* 3. Gases de efeito estufa. 4. Mudança do uso da terra. 5.Savana. I. Gatto, Alcides, II. Oliveira, Alexsandra Duarte de.

## REFERÊNCIA BIBLIOGRÁFICA

RIBEIRO, F. P. (2018). Dinâmica da serapilheira e fluxos de Gases de Efeito Estufa em plantios de eucalipto e vegetação nativa do Cerrado. Tese de Doutorado em Ciências Florestais, Publicação PPGEFL.TD-089/2018. Programa de Pós-graduação em Ciências Florestais, Universidade de Brasília, Brasília, DF, 83p.

## CESSÃO DE DIREITOS

AUTORA: Fabiana Piontekowski Ribeiro

TÍTULO: DINÂMICA DA SERAPILHEIRA E FLUXOS DE GASES DE EFEITO ESTUFA EM PLANTIOS DE EUCALIPTO E VEGETAÇÃO NATIVA DO CERRADO.

GRAU: Doutora ANO: 2018



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*"Não é o mais forte que sobrevive, nem o mais inteligente, mas o que melhor se adapta às mudanças".*

**(Leon Megginson)**

*A Deus que me deu a oportunidade de ser Eng. Florestal e estudar tudo que mais amo; A todas as pessoas que tem compromisso com as questões ambientais; Aos meus professores; e Ao meu irmão Fábio Campos Ribeiro, por ser minha razão infinita de querer continuar, por acreditar que nem mesmo o tempo pode apagar nosso amor... Enfim pelo simples fato de existir e me fazer tão feliz.*

**DEDICO**

AGRADECIMENTOS

Agradeço a DEUS, o maior cientista do universo, Dono de toda grandeza e sabedoria. Tu és a própria vida!

Ao meu orientador, professor Dr. Alcides Gatto que me acompanhou desde 2012 quando entrei no mestrado, sou muito agradecida por todos os ensinamentos.

A minha orientadora Alexsandra Duarte de Oliveira que foi um exemplo de profissional, sempre muito disponível, exigente, correta.

Aos pesquisadores: Arminda Moreira de Carvalho, Eloisa Aparecida Belleza Ferreira, Karina Pulrolnik e Sebastião Pires Neto pela gentileza e toda contribuição cientifica.

Ao Juaci Malakias agradeço pelo acompanhamento nos dados estatísticos. Quem não gostar do Juaci não gosta de mais ninguém do mundo.

A todos os profissionais da Embrapa Cerrados desde estagiário aos técnicos pela ajuda no laboratório e nas coletas. Adriano, Amanda, Artur, Aurea, Carlos, Carlão, Cid, Cintia, Diana, Euler, Fernanda Andrade, Fernanda Rodrigues, Francisco, Jacó, Julia, Lucas Cardoso, Lucas Vinícius, Lucas Vital, Márcia, Márcio, Mayara, Nelson, Paixão, Odenilza, Renato, Rhenilton, Valdecir, Vanessa, Vilderete.

Ao professor Dr. Adilson Jayme-Oliveira, pelos ensinamentos laboratoriais que foram de grande valia para essa pesquisa.

A Angela Bussinguer, por todo companheirismo durante esses seis anos de Pós-graduação, mais que uma amiga uma irmã, confidente, psicóloga, enfim sua amizade fez com que tudo se tornasse mais suave e bonito. Amo você!

Aos amigos do programa de pós-graduação Daniel, Danilo, Glauce, Fabrícia, Genilda, Gileno, Henrique, Karla, Ilvan, Isis, Lamartine, Ludovico, Marco Bruno e Maísa pela amizade e por toda ajuda ao longo da pesquisa.

Aos meus avós Elza Campos Ribeiro Oliveira e José Ribeiro Neto que desde sempre me incentivaram a estudar.

Aos meus pais Edina Campos Ribeiro (in memoriam) e Aldo Ribeiro (in memoriam) pelo dão da vida.

Ao meu tio Sula (in memoriam) eu penso que toda sua luta pela vida transformou meu coração.

A minha tia/irmã/mãe Rejane Campos Ribeiro grande incentivadora dos meus estudos desde da pré-escola até hoje. Grande personagem na realização dos meus sonhos. Minha gratidão por você será eterna. Amo você!

Ao meu irmão Fabio Campos Ribeiro, meu braço direito, meu companheiro para vida inteira. Amo você!

Ao meu dileto Valderli Jorge Piontekowski, o cara mais inteligente que já conheci. Obrigada por me dá o maior presente da minha vida, amo vocês!

Aos familiares que me incentivaram e ajudaram durante todo período de estudos.

Aos meus amigos da época da escola e da graduação em Engenharia Florestal na UFAC.

As minhas professoras da época da escola Kelly e Patrícia, que marcaram minha vida e são minhas amigas até hoje.

Aos professores do curso de Engenharia Florestal da Universidade Federal do Acre.

Aos professores do departamento de Engenharia Florestal da Universidade de Brasília do curso de Ciências Florestais.

Aos profissionais da UnB, do Departamento de Engenharia Florestal, Programa de Pós-Graduação em Ciências Florestais e Fazenda Água Limpa (FAL). Muito obrigada por tudo!

A CAPES pela concessão da Bolsa de Doutorado.

Enfim, a todos que contribuíram direta ou indiretamente para a realização desta pesquisa.

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### Metodologia Geral



### Capítulo 1

**Capítulo 2**

# 1. INTRODUÇÃO GERAL

Em âmbito global, evidências científicas apontam para graves consequências causadas pelo aumento da temperatura média global e da concentração de Gases de Efeito Estufa - GEE, principalmente dióxido de carbono ($CO_2$), metano ($CH_4$) e óxido nitroso ($N_2O$), provenientes de emissões antrópicas. Tal preocupação traz a necessidade de instituir medidas para conter o aumento da concentração desses gases na atmosfera (IPCC, 2007). A Convenção da ONU para o Clima (United Nations Convention on Climate Change) surgiu para dar suporte aos esforços conjuntos dos países membros, articulando-os e formulando metas a serem cumpridas a curto e médio prazos para redução das emissões de GEE no conjunto de suas economias (Acordo Copenhague, 2009).

O Brasil, numa ação voluntária, avançou consideravelmente, estabelecendo a Lei nº 12.187 de 29.12.2009, que instituiu a Política Nacional sobre Mudança do Clima – PNMC, exercendo liderança nas discussões sobre o mecanismo de Redução de Emissões por Desmatamento e Degradação (REDD) e sugerindo ações nacionais de redução das emissões de GEE. Essa ação foi traduzida na meta voluntária de redução das emissões até 2020, entre 36,1 % e 38,9 %, deixando de emitir cerca de 1 bilhão de toneladas de $CO_2$ (Brasil, 2009).

Outra ação do Governo brasileiro na mesma direção foi reconhecer a importância das florestas, com a publicação do Decreto nº 7.390/2010, no qual destaca-se a redução de 80 % de desmatamento na Amazônia e 40 % no Cerrado, com corte na emissão de 669 milhões de toneladas de $CO_2$ eq (Brasil, 2010). O Cerrado sempre foi um bioma importante para o desenvolvimento econômico brasileiro, principalmente no que se refere à produção de alimentos (Carneiro-Filho e Costa, 2016). Representando a savana mais biodiversa do mundo, suas fisionomias formam uma ecorregião única, composta de pastagens de savana e formações florestais (Castro et al., 2016). Sua vegetação apresenta cerca de 12.000 espécies de plantas descritas (Mendonça et al., 2008) onde 4.800 espécies de plantas e vertebrados endêmicos estão ameaçadas de extinção (Strassbur et al., 2017).

Apesar de sua relevância, o Bioma muitas vezes foi negligenciado nos debates nacionais e internacionais sobre mudanças climáticas. A atual taxa de conversão do Cerrado não é sustentável, liberando um volume de $CO_2$ não estimado anteriormente (Noojipady et al., 2017). O reconhecimento da importância do Cerrado por seu papel na mitigação das mudanças climáticas fez com que entre os planos setoriais de mitigação do compromisso voluntário fosse criado o Plano de Ação para Prevenção e Controle do Desmatamento e das

Queimadas no Cerrado (PPCerrado) que tem como foco principal a mitigação das GEE relacionadas às mudanças do uso da terra.

O compromisso assumido pelo Governo Federal propõe adotar, entre outras medidas: "ações de reflorestamento no país, expandindo a área reflorestada de 6,0 milhões de ha para 9,0 milhões de ha, contribuindo para a redução de 8 a 10 milhões de toneladas de $CO_2$ eq (Brasil, 2010). Todavia, uma das principais preocupações do setor florestal refere-se a como proceder o monitoramento das áreas florestadas e de seu potencial de fixação de $CO_2$ via atividade fotossintética e de mitigação na concentração de GEE na atmosfera. Assim, foram formuladas políticas públicas para incentivar projetos de inventários das emissões e remoções de GEE nos principais Biomas do País, bem como, propor estratégias de mitigação e adaptação para o enfrentamento dos impactos das mudanças climáticas, como signatário da Convenção do Clima (Saltus, 2014).

Da área total de árvores plantadas, 73 % são plantios de eucalipto. Em nível mundial, o Brasil está entre os principais produtores celulose, papel e painéis de madeira, com expressiva geração de empregos e renda em todo País (Ibá, 2017). Por sua relevância para o desenvolvimento social, ambiental e econômico, o setor florestal tem investido também para transformar subprodutos e resíduos dos processos industriais em produtos inovadores e que contribuam para o fortalecimento de uma economia de baixo carbono (C) (Ibá, 2017). Estima-se que, para atender o crescente aumento da população, em um cenário de baixo C, energias renováveis e desmatamento líquido zero, serão necessários 250 milhões de ha adicionais de florestas plantadas no mundo (Ibá, 2017).

Dessa forma, este estudo faz parte do projeto Saltus (Dinâmica da emissão de gases de efeito estufa e dos estoques de carbono em florestas brasileiras naturais e plantadas), uma iniciativa que aborda estudos para gerar informações consistentes sobre as florestas no tocante a estoques de carbono e emissão de gases de efeito estufa, visando cobrir as lacunas de informações desta natureza existentes no país, bem como avançar no sentido de refinar o uso de técnicas modernas de simulação nas estimativas de emissões nacionais de GEE.

A fixação de C pela vegetação nativa e sistemas florestais, bem como a redução de emissão de GEE pelas atividades agropecuárias e industriais, podem ser remuneradas ao contribuírem para a regulação climática. Todavia, indicadores ambientais como presença de serapilheira, decomposição e mineralização dos resíduos e estimativas do balanço de C são fundamentais para subsidiar o potencial desses mecanismos de valoração e políticas públicas relacionadas à temática ambiental. A remuneração desses serviços pode também contribuir

na conservação de áreas de Cerrado natural, recuperação de áreas nativas degradadas e na ampliação de florestas plantadas nas áreas que já se encontram desmatadas.

## 1.1 QUESTÕES DE PESQUISA

Como as florestas nativas e os plantios de eucalipto se comportam em relação aos GEE? Emitem? Quanto? Qual é a posição do Bioma Cerrado no cenário mundial? Qual o desempenho da ciclagem de nutrientes e as suas relações com os fluxos de GEE em plantios de eucalipto e vegetação nativa no Cerrado?

## 1. 2 OBJETIVOS

### a) Objetivo geral

Avaliar a dinâmica da serapilheira e dos fluxos de GEEs ($CH_4$ e $N_2O$) em plantios de eucalipto com diferentes idades e vegetação nativa do cerrado, no Distrito Federal.

### b) Objetivos específicos

- Avaliar a dinâmica da serapilheira (produção, estoque e decomposição) e o efeito da sazonalidade;
- Quantificar a biomassa e o carbono na serapilheira estocada e a composição química e os nutrientes da serapilheira remanescente;
- Quantificar os fluxos de metano ($CH_4$) e óxido nitroso ($N_2O$) do solo; e disponibilidade do nitrogênio na forma de nitrato ($NO_3^-$) e amônio ($NH_4^+$);
- Identificar variáveis ambientais e meteorológicas controladoras da dinâmica da serapilheira e dos fluxos de GEE em plantios de eucalipto com diferentes idades e vegetação nativa do cerrado.

Para atingir os objetivos propostos, a pesquisa da tese foi estruturada em dois temas e capítulos. Cada capítulo encontra-se na forma de artigo, conforme apresentado a seguir:

**Capítulo 1 - "Litter dynamics in *Eucalyptus* and native forest ecosystems in the Brazilian Cerrado"**

**Capítulo 2 - "$CH_4$ and $N_2O$ fluxes in planted forests and native ecosystems in the Brazilian Cerrado"**

# 2. METODOLOGIA GERAL

## 2.1. ÁREA DE ESTUDO

O estudo foi conduzido no Cerrado da região central do Brasil (Planalto Central) no Núcleo Rural Quebrada dos Neres, Paranoá, Distrito Federal (Figura 1).

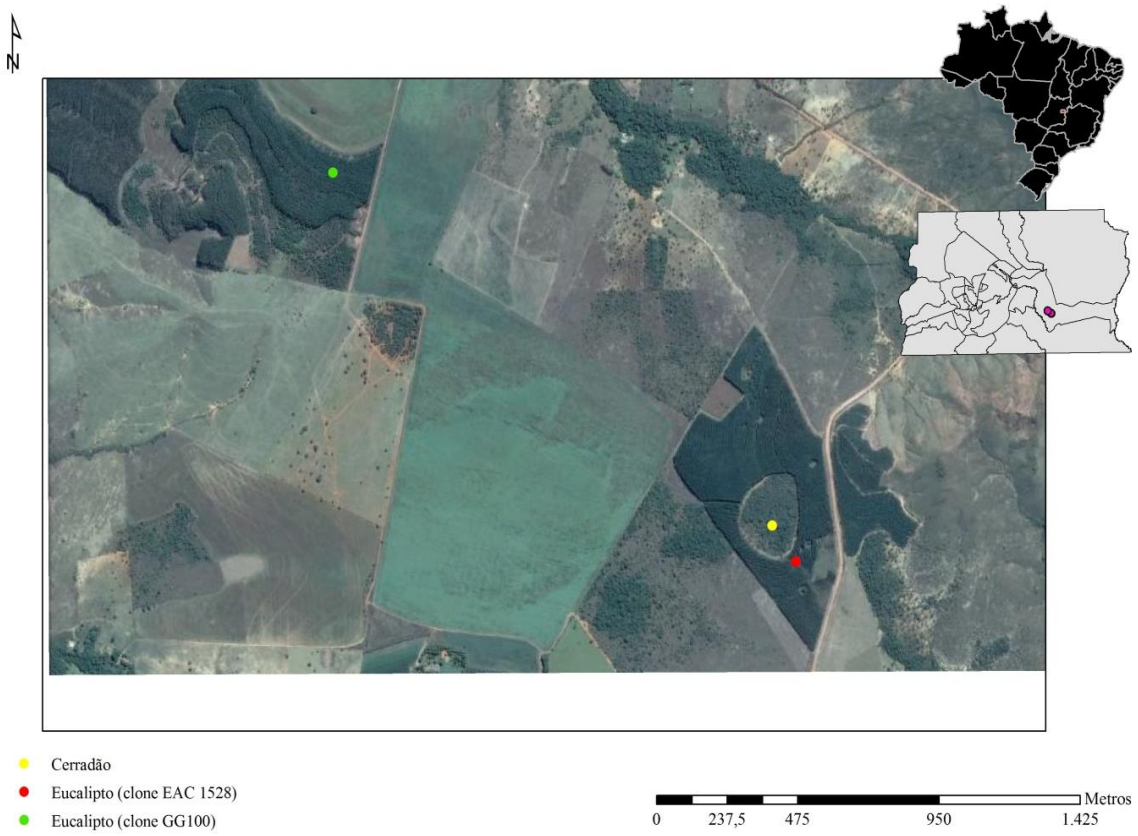


**Figura 1 -** Localização das áreas de estudo, núcleo rural Quebrada dos Neres, Paranoá, Distrito Federal, Brasil. Fonte: IBGE (2009).

As áreas de estudo consistem de: vegetação nativa de cerrado (CE) com 3,5 ha e áreas com dois povoamentos de eucalipto (híbrido *Eucalyptus urophylla* × *Eucalyptus grandis*) com 12 e 19 ha plantados com os clones EAC 1528 (E1) e GG100 (E2), respectivamente. As características gerais das áreas estudadas estão apresentadas na Tabela 1.

**Tabela 1** - Descrição e histórico das áreas de estudo, Paranoá, Distrito Federal, Brasil.

| Áreas | Símbolo | Descrição |
|---|---|---|
|  | CE | Área com vegetação nativa de cerrado qualificada como Cerradão que representa formação florestal com aspectos xeromórficos, dossel predominantemente contínuo e cobertura arbórea de 50 a 90 %. A altura média do estrato arbóreo varia de 8 a 15 m. (Ribeiro e Walter, 2008). As coordenadas são: 15º 53' S, 47º 38' W e altitude de 930 m. Com histórico de incêndio anterior ao ano de 1996. |
|  | E1 | Povoamento clonal de eucalipto, nas coordenadas (15º 53'S, 47º 39' W e altitude de 948 m). Implantado em dezembro de 2011, no espaçamento de 3,5 × 1,7 m. Anteriormente possuia vegetação nativa de Cerrado. Para o estabelecimento do plantio, o solo foi revolvido com grade aradora em faixa de 15 cm e subsolagem na linha de plantio a 90 cm de profundidade. Diâmetro a altura do peito (DAP) médio das árvores foi de 13,4; 14,6 e 15,5 cm, e altura média de 19,4, 22,6 e 23,0 m nos anos de 2014, 2015 e 2016, respectivamente. |
|  | E2 | Povoamento clonal de eucalipto, nas coordenadas (15º 53'S, 47º 38' W e altitude de 946 m). Implantado em dezembro de 2009, com espaçamento de 3,5 × 1,7 m. O histórico da área era de uso agrícola, sendo utilizada entre 2003 e 2005 para o cultivo de soja, em 2006 para o cultivo de sorgo e, no período de 2007 a 2009, foi novamente cultivado com soja. O preparo do solo consistiu em revolvimento com grade aradora em faixas de 15 cm e uso do sulcador no centro da faixa a 25 cm de profundidade. DAP médio das árvores de 15,4, 16,5 e 17,2 cm e a altura média 24,4; 28,5 e 30,0 m nos anos de 2014, 2015 e 2016, respectivamente. |

Nas áreas durante a implantação com eucalipto (E1 e E2) foi realizada a correção da acidez do solo com a aplicação e incorporação de calcário dolomítico (2,5 t $ha^{-1}$) a 20 cm de profundidade, e após dois meses foi colocado 700 kg $ha^{-1}$ de gesso agrícola na superfície do solo. A adubação de plantio consistiu em 200 g por planta de NPK (5-25-15). Após um ano da implantação dos povoamentos (2010 e 2012), procedeu-se a adubação de cobertura com 60 kg $ha^{-1}$ de $K_2O$ na forma de cloreto de potássio, 50 kg $ha^{-1}$ de N na forma de ureia e 1 g de boro por planta na forma de bórax. Em janeiro de 2014, fez-se nova aplicação de 60 kg $ha^{-1}$ de $K_2O$.

O solo das áreas é classificado como Latossolo Vermelho-Amarelo distrófico, textura muito argilosa, com teor de argila ≥ 60 % (Embrapa, 2013). Os atributos químicos do solo (0-5 e 5-10 cm de profundidade) estão apresentados na Tabela 2.

**Tabela 2** - Atributos químicos do solo nas profundidades de 0-5 e 5-10 cm, nas três áreas estudadas, no Distrito Federal, Brasil.

| **Atributos**[(1)] | | **E1** | **E2** | **CE** | **E1** | **E2** | **CE** |
|---|---|---|---|---|---|---|---|
| | | ---------- 0-5 cm ---------- | | | ---------- 5-10 cm ---------- | | |
| MO | dag $kg^{-1}$ | 3,3 | 3,0 | 3,8 | 2,9 | 2,6 | 3,2 |
| pH | ($H_2O$) | 5,0 | 5,4 | 5,1 | 4,9 | 5,3 | 5,1 |
| P* | mg $dm^{-3}$ | 1,5 | 5,3 | 1,8 | 1,4 | 3,5 | 1,2 |
| H+Al | mg $dm^{-3}$ | 8,2 | 7,0 | 9,5 | 8,3 | 7,1 | 8,6 |
| SB | mg $dm^{-3}$ | 1,8 | 4,3 | 1,3 | 1,6 | 2,6 | 0,8 |
| CTC-T | mg $dm^{-3}$ | 10,0 | 11,3 | 10,8 | 9,8 | 9,7 | 9,5 |
| V | % | 17,7 | 38,0 | 11,8 | 15,8 | 26,7 | 8,7 |
| B | mg $kg^{-1}$ | 0,5 | 1,1 | 0,5 | 0,5 | 0,9 | 0,5 |
| Zn | mg $L^{-1}$ | 0,3 | 0,6 | 0,6 | 0,3 | 1,3 | 0,4 |

[(1)] MO: matéria orgânica do solo, método Walkley-Black; pH em água, relação solo:solução 1:2,5; P e Zn: extrator Mehlich-1; H+Al: acidez potencial, extrator acetato de cálcio 0,5 mol $L^{-1}$, pH 7; SB: soma de bases; V: índice de saturação por bases; B: extrator água quente. E1 e E2: área com povoamento clonal de eucalipto; CE: área com vegetação nativa do Cerrado.

O clima da região é do tipo Aw tropical chuvoso segundo o sistema de classificação de Köppen, com duas estações climáticas bem definidas: seca, entre os meses de maio a setembro, e chuvosa, entre outubro e abril. A temperatura média do ar foi de 22, 22, e 21 ºC, e a precipitação pluvial total anual foram de 1311, 1052 e 1235 mm, para os anos 2014, 2015, e 2016, respectivamente (Figura 2), segundo a base de dados da Estação Climatológica da Empresa de Assistência Técnica e Extensão Rural do Distrito Federal (Emater-DF).

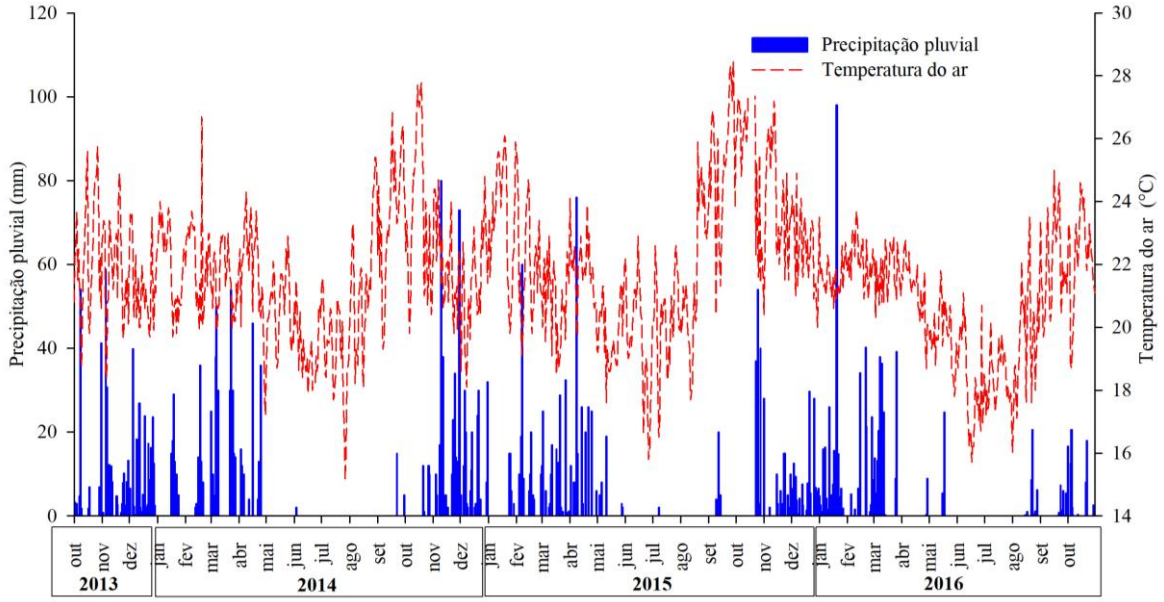


**Figura 2 -** Precipitação pluvial (mm) e temperatura do ar (ºC) de outubro de 2013 a outubro de 2016, Paranoá, Distrito Federal, Brasil. Fonte: Emater-DF

Para o estudo da composição florística foi realizado um inventário florestal. A área da vegetação lenhosa foi amostrada em parcelas retangulares de 20 × 50 cm, georreferenciada e lançadas no sistema de amostragem inteiramente casualizado com auxílio do programa o ArcMap, que é a aplicação central do ArcGIS, versão 9.3. O inventário florístico foi realizado na estação seca (agosto 2015), sendo selecionadas aleatoriamente 10 parcelas de 0,1 ha.

Em cada parcela amostrada, todos os indivíduos lenhosos arbóreos e arbustivos, vivos e mortos em pé, com Db (diâmetro tomado a 30 cm do nível do solo) igual ou superior a 5 cm (Felfili et al., 2005) foram identificados botanicamente e as variáveis Db e altura total, registradas. A área de formação florestal (Cerradão) apresentou densidade de 1.858 ind $ha^{-1}$, distribuídos em 83 espécies e 41 famílias botânicas, com Db ≥ 5 cm (Tabela 3).

**Tabela 3 -** Parâmetros fitossociológicos das espécies lenhosas (Db ≥ 5 cm) em área de formação florestal (Cerradão), Núcleo Rural Quebrada dos Neres, Paranoá, Distrito Federal, Brasil.

| Espécie | Família | DA | DR | FA | FR | DoA | DoR | IVI* |
|---|---|---|---|---|---|---|---|---|
| *Miconia pohliana* | Melastomataceae | 290 | 15,61 | 100 | 2,92 | 3,12 | 18,45 | 36,98 |
| *Qualea grandiflora* | Vochysiaceae | 161 | 8,67 | 100 | 2,92 | 2,59 | 15,31 | 26,90 |
| *Xylopia aromatica* | Annonaceae | 171 | 9,20 | 100 | 2,92 | 1,41 | 8,32 | 20,45 |
| *Miconia albicans* | Melastomataceae | 230 | 12,38 | 100 | 2,92 | 0,83 | 4,92 | 20,22 |
| *Alibertia edulis* | Rubiaceae | 137 | 7,37 | 100 | 2,92 | 0,42 | 2,50 | 12,80 |
| *Kielmeyera coriacea* | Clusiaceae | 121 | 6,51 | 100 | 2,92 | 0,54 | 3,21 | 12,65 |
| *Qualea parviflora* | Vochysiaceae | 24 | 1,29 | 80 | 2,34 | 1,05 | 6,20 | 9,83 |
| *Emmotum nitens* | Icacinaceae | 45 | 2,42 | 100 | 2,92 | 0,70 | 4,15 | 9,50 |
| *Xylopia brasiliense* | Annonaceae | 76 | 4,09 | 100 | 2,92 | 0,37 | 2,19 | 9,20 |
| *Astronium fraxinifolium* | Anacardiaceae | 56 | 3,01 | 100 | 2,92 | 0,48 | 2,86 | 8,80 |
| *Curatella americana* | Dilleniaceae | 34 | 1,83 | 90 | 2,63 | 0,62 | 3,69 | 8,15 |
| *Amaioua guianensis* | Rubiaceae | 31 | 1,67 | 90 | 2,63 | 0,51 | 3,04 | 7,34 |
| *Rudgea viburnoides* | Rubiaceae | 40 | 2,15 | 100 | 2,92 | 0,30 | 1,76 | 6,84 |
| *Byrsonima pachyphylla* | Malpighiaceae | 32 | 1,72 | 100 | 2,92 | 0,21 | 1,24 | 5,89 |
| *Terminalia agentea* | Combretaceae | 23 | 1,24 | 70 | 2,05 | 0,42 | 2,47 | 5,76 |
| *Eriotheca pubescens* | Malvaceae | 12 | 0,65 | 70 | 2,05 | 0,33 | 1,97 | 4,66 |
| *Davila elliptica* | Dilleniaceae | 24 | 1,29 | 80 | 2,34 | 0,17 | 0,99 | 4,62 |
| *Simarouba versicolor* | Simaroubaceae | 20 | 1,08 | 80 | 2,34 | 0,19 | 1,13 | 4,55 |
| *Schefflera macrocarpa* | Araliaceae | 16 | 0,86 | 80 | 2,34 | 0,18 | 1,04 | 4,24 |
| *Piptocarpha rotundifolia* | Asteraceae | 19 | 1,02 | 70 | 2,05 | 0,12 | 0,71 | 3,78 |
| *Eugenia dysenterica* | Myrtaceae | 17 | 0,91 | 50 | 1,46 | 0,20 | 1,17 | 3,55 |
| *Alibertia sessilis* | Rubiaceae | 20 | 1,08 | 70 | 2,05 | 0,07 | 0,42 | 3,54 |
| *Acosmium dasycarpum* | Fabaceae | 13 | 0,70 | 80 | 2,34 | 0,07 | 0,44 | 3,48 |
| *Blepharocalyx salicifolius* | Myrtaceae | 25 | 1,35 | 40 | 1,17 | 0,13 | 0,77 | 3,28 |
| *Ouratea grandiflora* | Ochnaceae | 9 | 0,48 | 60 | 1,75 | 0,14 | 0,85 | 3,09 |
| *Virola sebifera* | Myristicaea | 9 | 0,48 | 60 | 1,75 | 0,09 | 0,52 | 2,76 |
| *Roupala montana* | Proteaceae | 20 | 1,08 | 30 | 0,88 | 0,12 | 0,73 | 2,68 |
| *Machaerium opacum* | Papilionoideae | 8 | 0,43 | 50 | 1,46 | 0,10 | 0,58 | 2,48 |
| *Byrsonima laxiflora* | Malpighiaceae | 11 | 0,59 | 50 | 1,46 | 0,06 | 0,34 | 2,40 |
| *Brosimum gaudichaudii* | Moraceae | 10 | 0,54 | 50 | 1,46 | 0,04 | 0,22 | 2,22 |
| *Psidium laruotteanum* | Myrtaceae | 8 | 0,43 | 50 | 1,46 | 0,04 | 0,24 | 2,14 |
| *Rapanea guianensis* | Myrsinaceae | 10 | 0,54 | 40 | 1,17 | 0,07 | 0,41 | 2,12 |
| *Erythroxylum decidumm* | Erythroxylaceae | 6 | 0,32 | 50 | 1,46 | 0,05 | 0,27 | 2,05 |
| *Strychnos pseudoquina* | Loganiaceae | 4 | 0,22 | 40 | 1,17 | 0,11 | 0,64 | 2,03 |
| *Diospyros burchellii* | Ebenaceae | 6 | 0,32 | 50 | 1,46 | 0,04 | 0,23 | 2,02 |
| *Tocoyena formosa* | Rubiaceae | 6 | 0,32 | 50 | 1,46 | 0,03 | 0,15 | 1,94 |
| *Connarus fulvus* | Connaraceae | 9 | 0,48 | 40 | 1,17 | 0,05 | 0,27 | 1,92 |
| *Psidium firmum* | Myrtaceae | 6 | 0,32 | 40 | 1,17 | 0,06 | 0,36 | 1,86 |
| *Erythroxylum daphnites* | Erythroxylaceae | 5 | 0,27 | 40 | 1,17 | 0,04 | 0,21 | 1,65 |
| *Lafoensia pacari* | Lythraceae | 5 | 0,27 | 40 | 1,17 | 0,02 | 0,14 | 1,58 |
| *Symplocus rhamnifolia* | Symplocaceae | 4 | 0,22 | 30 | 0,88 | 0,06 | 0,35 | 1,44 |
| *Tapirira guianensis* | Anacardiaceae | 3 | 0,16 | 30 | 0,88 | 0,06 | 0,37 | 1,40 |
| *Terminalia fagifolia* | Combretaceae | 3 | 0,16 | 30 | 0,88 | 0,04 | 0,24 | 1,28 |
| *Salvertia convallariaeodora* | Vochysiaceae | 4 | 0,22 | 20 | 0,58 | 0,08 | 0,45 | 1,25 |
| *Dimorphandra mollis* | Mimosoideae | 6 | 0,32 | 20 | 0,58 | 0,05 | 0,29 | 1,19 |
| *Myrcia tomentosa* | Myrtaceae | 4 | 0,22 | 30 | 0,88 | 0,02 | 0,10 | 1,19 |
| *Erythroxylum suberosum* | Erythroxylaceae | 2 | 0,11 | 20 | 0,58 | 0,05 | 0,27 | 0,97 |
| *Aegiphila lhotzkiana* | Lamiaceae | 3 | 0,16 | 20 | 0,58 | 0,02 | 0,10 | 0,85 |
| *Byrsonima intermedia* | Malpighiaceae | 3 | 0,16 | 20 | 0,58 | 0,01 | 0,08 | 0,83 |
| *Ouratea hexasperma* | Ochnaceae | 3 | 0,16 | 20 | 0,58 | 0,01 | 0,08 | 0,83 |
| *Gomidesia spectabilis* | Myrtaceae | 3 | 0,16 | 20 | 0,58 | 0,01 | 0,08 | 0,82 |
| *Miconia ferruginata* | Melastomataceae | 3 | 0,16 | 20 | 0,58 | 0,01 | 0,06 | 0,81 |

Continua...

**Tabela 3** - Continuação.

| Espécie | Família | DA | DR | FA | FR | DoA | DoR | IVI* |
|---|---|---|---|---|---|---|---|---|
| *Stryphnodendron adstringens* | Fabaceae | 2 | 0,11 | 20 | 0,58 | 0,01 | 0,09 | 0,78 |
| *Hirtella gracilipes* | Chrysobalanaceae | 2 | 0,11 | 20 | 0,58 | 0,01 | 0,04 | 0,74 |
| *Cecropia pachystachya* | Urticaceae | 3 | 0,16 | 10 | 0,29 | 0,04 | 0,24 | 0,70 |
| *Vochysia tucanorum* | Vochysiaceae | 2 | 0,11 | 10 | 0,29 | 0,04 | 0,22 | 0,62 |
| *Enterolobium contortisiliquum* | Fabaceae | 1 | 0,05 | 10 | 0,29 | 0,04 | 0,25 | 0,60 |
| *Byrsonima coccolobifolia* | Malpighiaceae | 3 | 0,16 | 10 | 0,29 | 0,02 | 0,14 | 0,59 |
| *Pseudobombax grandiflorum* | Malvaceae | 3 | 0,16 | 10 | 0,29 | 0,02 | 0,12 | 0,57 |
| *Machaerium acutifolium* | Papilionoideae | 2 | 0,11 | 10 | 0,29 | 0,03 | 0,16 | 0,56 |
| *Caryocar brasiliense* | Caryocaraceae | 2 | 0,11 | 10 | 0,29 | 0,02 | 0,14 | 0,54 |
| *Mamuria dianese* | Melastomataceae | 2 | 0,11 | 10 | 0,29 | 0,02 | 0,12 | 0,52 |
| *Dalbergia foliolosa* | Fabaceae | 2 | 0,11 | 10 | 0,29 | 0,02 | 0,10 | 0,50 |
| *Gomidesia lindeniana* | Myrtaceae | 2 | 0,11 | 10 | 0,29 | 0,01 | 0,08 | 0,48 |
| *Ficus adhatodifolia* | Moraceae | 1 | 0,05 | 10 | 0,29 | 0,01 | 0,08 | 0,43 |
| *Annona crassiflora* | Annonaceae | 1 | 0,05 | 10 | 0,29 | 0,01 | 0,08 | 0,43 |
| *Byrsonima verbascifolia* | Malpighiaceae | 1 | 0,05 | 10 | 0,29 | 0,01 | 0,07 | 0,41 |
| *Diospyros sericea* | Ebenaceae | 1 | 0,05 | 10 | 0,29 | 0,01 | 0,05 | 0,40 |
| *Erythroxylum camprestre* | Erythroxylaceae | 1 | 0,05 | 10 | 0,29 | 0,01 | 0,05 | 0,39 |
| *Syagrus comosa* | Arecaceae | 1 | 0,05 | 10 | 0,29 | 0,01 | 0,05 | 0,39 |
| *Zanthoxylum rhoifolium* | Rutaceae | 1 | 0,05 | 10 | 0,29 | 0,01 | 0,04 | 0,38 |
| *Eremanthus erythropappus* | Asteraceae | 1 | 0,05 | 10 | 0,29 | 0,00 | 0,03 | 0,38 |
| *Tabebuia caraiba* | Bignoniaceae | 1 | 0,05 | 10 | 0,29 | 0,00 | 0,03 | 0,38 |
| *Vochysia rufa* | Vochysiaceae | 1 | 0,05 | 10 | 0,29 | 0,00 | 0,03 | 0,37 |
| *Byrsonima klacia* | Malpighiaceae | 1 | 0,05 | 10 | 0,29 | 0,00 | 0,02 | 0,37 |
| *Guapira noxia* | Nyctaginaceae | 1 | 0,05 | 10 | 0,29 | 0,00 | 0,02 | 0,37 |
| *Rourea induta* | Connaraceae | 1 | 0,05 | 10 | 0,29 | 0,00 | 0,02 | 0,37 |
| *Salacia crassifolia* | Celastraceae | 1 | 0,05 | 10 | 0,29 | 0,00 | 0,02 | 0,36 |
| *kielmeyera speciosa* | Clusiaceae | 1 | 0,05 | 10 | 0,29 | 0,00 | 0,02 | 0,36 |
| *Connarus suberosos* | Connaraceae | 1 | 0,05 | 10 | 0,29 | 0,00 | 0,01 | 0,36 |
| *Norantea guianensis* | Marcgraviaceae | 1 | 0,05 | 10 | 0,29 | 0,00 | 0,01 | 0,36 |
| *Ouratea parviflora* | Ochnaceae | 1 | 0,05 | 10 | 0,29 | 0,00 | 0,01 | 0,36 |
| *Casearia sylvestris* | Flacourtiaceae | 1 | 0,05 | 10 | 0,29 | 0,00 | 0,01 | 0,36 |
| **Total** | | **1858** | **100** | **3420** | **100** | **16.91** | **100** | **300** |

DA = Densidade absoluta (N $ha^{-1}$); DR = Densidade relativa (%); FA = Frequência absoluta (%); FR = Frequência relativa (%); DoA = Dominância Absoluta ($m^2$ $ha^{-1}$); DoR = Dominância Relativa (%) e IVI = Índice de Valor de Importância (%). *Espécies em ordem decrescente de IVI.

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# CAPÍTULO 1

## LITTER DYNAMICS IN *EUCALYPTUS* AND NATIVE FOREST ECOSYSTEMS IN THE BRAZILIAN CERRADO

**RESUMO**

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**Brasília, 23 de março de 2018**

A dinâmica da serapilheira está relacionada aos fatores de sua formação, acúmulo e decomposição. Por vezes, em um mesmo ecossistema a dinâmica da serapilheira pode variar ao longo do tempo, de acordo com as condições edafoclimáticas de cada região. O objetivo desse trabalho foi avaliar a dinâmica de serapilheira nas épocas do ano em três áreas: formação florestal Cerradão - CE e em dois povoamentos de híbridos de *Eucalyptus urophylla* x *Eucalyptus grandis* com diferentes idades: E1 (34-58 meses) e E2 (58-82 meses). Para isso, procederam as coletas da serapilheira produzida, estocada e sua massa remanescente, ao longo de 720 dias. A avaliação da massa remanescente de serapilheira, em cada área foi realizada a partir da distribuição aleatória de 648 litter bags sobre o solo. Foram realizadas análises químicas (N, P, C) e dos componentes estruturais da parede celular (lignina, celulose e hemicelulose) da serapilheira remanescente. Também foi determinado o C estocado na serapilheira. Como principais resultados foi verificado efeito da sazonalidade na produção de serapilheira. Para ambos os anos de avaliação foi observada maior biomassa e C na serapilheira no E2. Em contrapartida, a maior taxa de decomposição foi para o CE, especialmente no segundo ano de avaliação (massa remanescente aos 720 dias de 35 %, 37 % e 23 % para E1, E2 e CE, respectivamente), o que foi atribuído a maior liberação aparente de N, umidade do solo e biodiversidade na área nativa. Os teores de lignina aumentaram, os de celulose diminuíram e os de hemicelulose ficaram estáveis ao longo dos 720 dias, sugerindo que a decomposição da celulose é proporcional à perda de serapilheira e que a resistência à decomposição de lignina ocorre pelo menos até dois anos de avaliação. Também foi observado um aumento na concentração dos nutrientes N e P da massa remanescente e correlações positivas entre massa remanescente e as relações C:N e C:P. A relação C:N da serapilheira foi ≥ 76:1 no tempo 0 e ≥ 30:1 aos 720 dias para as três áreas. Os resultados do presente estudo reforçam a importância de pesquisas de longo prazo principalmente para decomposição de serapilheiras em ecossistemas florestais.

**Palavras chave:** Savana tropical, massa remanescente, qualidade da serapilheira, híbridos de *Eucalyptus urophylla* x *Eucalyptus grandis.*

**ABSTRACT**

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Litter dynamics are related to the factors of its formation, accumulation and decomposition. Sometimes, in a same ecosystem the dynamics of the litter can vary over time, according to the edaphoclimatic conditions of each region. The aim of this study was to evaluate the litter dynamics in the seasons of the year in three areas: forest formation Cerradão – CE, and in two hybrids stands of *Eucalyptus urophylla* x *Eucalyptus grandis* with different ages: E1 (34-58 months) and E2 (58-82 months). For this, the produced litter, stored litter and the remaining mass was collected over 720 days. The evaluation of the remaining litter mass in each area was performed from the random distribution of 648 litter bags on the soil. Chemical analyzes (N, P, C) and the structural components of the cell wall (lignin, cellulose and hemicellulose) of the remaining litter were performed. The C of the litter stock was also determined. The main results showed seasonal effect on litter production. For both years of evaluation were observed higher biomass and C content in litter at E2. In contrast, the highest decomposition rate was for the CE, especially in the second year of evaluation (mass remaining at 720 days of 35 %, 37 % and 23 % for E1, E2 and CE, respectively), attributed to the higher apparent liberation of N, soil moisture and biodiversity in the native area. Lignin contents increased, cellulose decreased, and hemicellulose remained stable throughout the 720 days, suggesting that the cellulose decomposition is proportional to the loss of litter and that resistance to lignin decomposition occurs at least up to two years of evaluation. It was also observed an increase in the N and P nutrients concentration of the remaining mass and positive correlations among the remaining mass and the C:N and C:P ratios. The C:N ratio of litter was ≥ 76:1 at time 0 and ≥ 30:1 at 720 days for the three areas. The results of the present study reinforce the importance of long term research, mainly for the decomposition of litter in forest ecosystems.

**Keywords:** Tropical savanna; Remaining mass; Litter quality; Hybrids of *Eucalyptus urophylla* x *Eucalyptus grandis.*

# 1. INTRODUCTION

Nutrient cycling in forest ecosystems is controlled primarily by climate, site, abiotic properties (topography, parent material), biotic communities and human activity (harvesting, fertilization) (Osborne et al., 2017; Fonseca et al., 2018; Fujji et al., 2018). In the context of sustainable production and environmental conservation, the maintenance of native forests and forest plantations is of great importance at local and global scale regulation of biogeochemical cycles (León and Osorio, 2014; Atwell et al., 2017).

In terrestrial ecosystems, litterfall represents the first phase of vegetation-to-soil transference of carbon and nutrient pools (Vitousek and Sanford, 1986). Thereafter, decomposition is an important process of nutrient return to the soil (Freschet et al., 2013; Hobbie, 2015). Nutrient availability and relations are widely considered decisive factors for litter decomposition, especially early stages of decay (Laclau et al., 2013; Prescott e Gray, 2013; van Huysen et al., 2013; Bachega et al., 2016; Ferreira et al., 2016a).

Factors such as temperature and precipitation are extremely relevant for litter decomposition and incorporation into the soil-plant system (Li et al., 2013; Zhang et al., 2014; Santos et al., 2017), aside from C compounds such as lignin, cellulose and hemicellulose, which can act as regulators of soil nutrient fluxes (Swift et al., 1979; Brow e Chang, 2014; Ferreira et al., 2016b). Although climate greatly influences the biogeochemical cycles, it has been verified that, not only C, but also the concentrations of nitrogen (N) and phosphorus (P) affect residue mineralization and have been used as quality parameters to explain decomposition rates in different systems, including forests plantations (Prescott and Gray, 2013; Bachega et al., 2016).

In recent decades' literature has pointed out patterns and mechanisms related to leaf litter dynamics, such as litter fall, standing stocks, decomposition, increases in N and refractory compounds with decomposition time (Berg, 2014; Viera et al., 2014; Inkotte et al., 2015; Turner et al., 2016; Ribeiro et al., 2017; Demi et al., 2018). However, most of studies do not address jointly litterfall, litter layer and litter decomposition or litter dynamics assessments associated with changes in soil use. As it is also the main input of nutrients into the system, litter also contributes to carbon stocks, controls the fluxes of greenhouse gases (GHG) and defines ecosystems structural and functional patterns. Determination of net responses of ecosystems to environmental changes, therefore, requires monitoring of ecosystem processes under natural field conditions.

In keeping with the needs for developing science-based policy recommendations for climate change mitigation the collection of data in the different compartments includes: effects of land-use change on below and above-ground productivity and carbon/nutrients budgets of forests is fundamental to know the level of GHG emissions and its main sources (IPCC, 2007).

Since litter plays a critical role in the account for change in land use in order to fulfill the interactive research needs for the most accurate predictions we formulated the following hypothesis: in the Cerrado, quantity, quality and litter decomposition occurs due to environmental conditions, forest type (native x planted) and age of eucalyptus plantations.

To investigate the litter dynamics in these different conditions, the objectives of this study were: (1) to quantify litterfall and litter layer as a function of seasonality; (2) Quantify the biomass and carbon stocks in the litter layer; (3) determine the decomposition rate and litter composition in terms of structural components (lignin, cellulose and hemicellulose) of the cell wall; and (4) to analyze the C, N and P concentrations of the remaining litter mass and their relationship with decomposition rates.

# 2. MATERIAL AND METHODS

## 2.1. STUDY SITE

See the description in general methodology (page 4).

## 2.2. LITTER ANALYSIS

To estimate litterfall between October 2014 and September 2016, 24 litter collectors of 0.25 $m^2$ were randomly placed in each of the three studied areas, fixed to a framework 50 cm above the soil surface, for monthly collections (Figure 1.1). To quantify the amount of forest litter deposited on the soil (Litter layer), nine random samples were taken, in two samplings per season (dry and rainy season), using a metallic 0.25 $m^2$ frame, laid loosely on the forest floor at a distance of at least five meters between the evaluated points.

**Figure 1.1 -** Frame of the litter collector.

The samples of litterfall were analyzed for seasonality, considering the dry and rainy seasons. The annual yields of litterfall and litter layer per area (dry mass) were calculated by the following equations:

$$L = ((\Sigma Y \times 10^4) / As / 10^3) \quad (1)$$

$$Ll = ((Z \times 10^4) / As / 10^6) \quad (2)$$

where L = Litter yield (kg $ha^{-1}year^{-1}$); Y = monthly litterfall (g $m^{-2}$ $month^{-1}$); As Sampling area (0. 25$m^2$); Ll = Litter layer (Mg $ha^{-1}year^{-1}$); Z = litter layer per season (g $m^{-2}$).

For the evaluation of the remaining litter mass per area, 648 litter bags (20 x 30 cm, made of 2 mm nylon mesh) were randomly distributed on the forest floor (Figure 1.2). The initial weight per litter per bag was 20 g of dry material (adapted from Santos and Withford, 1981). From October 2014 to September 2016, every three months 27 litter bags were collected and analyzed.

**Figure 1.2 -** Litter bag for litter decomposition analysis.

The litter was packed in labeled paper bags, sealed and sent to the laboratory to determine the fresh weight. Thereafter, the material was dried to constant weight in a forced air circulation oven (at 65 °C for 72 h) and then weighed again to determine dry weight.

The sequential method adapted from Robertson and Van Soest (1981) was used to analyze the structural composition of the cell wall of the remaining mass in the litter bags. The ground material was analyzed for neutral detergent fiber (NDF), acid detergent fiber (ADF) and crude lignin (L). The hemicellulose and cellulose contents were calculated, respectively, as the difference between them (NDF minus ADF) and (ADF minus L).

Of the remaining mass and litter layer 1.0 g litter samples were ground in a mortar and sieved (0.2 mm), and subsequently the total C content was determined using a Vario MACRO cube Elementary analyzer (Elementar Analysensysteme, Hanau, Germany). The total C content was multiplied by the litter layer to obtain the carbon stock. The total nitrogen (N) concentration was determined by the Kjeldahl method, after sulfur digestion and phosphorus (P) was determined by molecular absorption spectrophotometry (Embrapa, 2009).

## 2.3. DETERMINATION OF SOIL MOISTURE

One composite sample which is made up of eight sub samples of equal volume was taken at each plot (0-10 cm layer). From each composite sample, a soil aliquot was removed

to measure soil moisture content. The moisture content (gravimetric method) was calculated as the difference among dry and fresh weight, according to the following equation:

$$m = Wf - Wd \ / Wd \quad (3)$$

where: Wf = fresh weight and Wd = dry weight and moisture m (g/g) expressed as percent m (%) = m*100 (Embrapa, 1997).

## 2.4. DATA PROCESSING AND ANALYSIS

The quantitative litter variables (litterfall, litter layer and litter decomposition) and qualitative variables (lignin, cellulose, hemicellulose, C:N and C:P) were tested for normality (Shapiro-Wilk), followed by analysis of variance (ANOVA). Subsequently, the means were compared by the Tukey test ($p < 0.05$) by software SISVAR version 5.6, to detect possible differences among the areas, with regard to seasonality and over time in each area.

The data of remaining litter mass and qualitative variables were compared among the areas for the sampling periods (0, 90, 180, 270, 360, 450, 540, 630, and 720 days), and analyzed for each area over time by regression analysis.

Litter decomposition per area was calculated as proposed by Santos and Whitford (1981), based on the decomposition percentage, where the remaining litter rate was determined as the difference between the initial total litter mass amount (100 %) and each rate per assessment period. In addition, models with one, two, three and four parameters were tested and the one that best fit the data was the exponential model of one parameter proposed by Olson (1963) was used to calculate the mass (k) decomposition:

$$y = A\ e^{-kt} \quad (4)$$

where y is the amount of remaining material after a period of time; A the weight of the material at time zero (t = 0); k the decomposition constant obtained by software Sigma Plot for Windows 12.5 based on the litter quantities in the bags; and t the time in days (720 d).

The decomposition constant (k) allows to calculate the time projection required for the disappearance of 95 % (t = 3 / k) and 99 % (t = 3 / k) (Olson, 1963). The remaining mass data together with the quantitative variables (lignin, cellulose, hemicellulose, and C:N and C:P ratio) were subjected to Pearson's statistical correlation analysis, followed by Student's t-test, using the free software R (version 3.2.2). The liberation apparent rate for N and P

content was calculated by the difference of those nutrients between times 0 and 720 days ($X_0 - X_{720} * 100$).

# 3. RESULTS

## 3.1. LITTERFALL AND LITTER LAYER

The evaluations between October 2014 and September 2016 showed differences in litterfall owing to seasonality in the areas E1 ($F = 17.87$, $p < 0.01$) and CE ($F = 5.05$, $p = 0.017$) in the dry season of 2015 (Table 1.1). Among the areas, there were no statistical differences in litterfall, except in the dry season of 2016, with higher litterfall in the CE area ($F = 17.30$, $p < 0.01$). Moreover, there were seasonal differences in the two study years, with an about 60 and 45 % higher litterfall in the dry seasons of 2015 and 2016 than in the rainy seasons (2014/15 and 2015/16), only in the E1 area, the total litterfall was higher in the second than in the first year (Table 1.1; $p = 0.02$).

**Table 1.1** - Means and standard deviation of litterfall per season (rainy and dry) and per year in *Eucalyptus* stands E1 (planted in 2011, clone EAC 1528) and E2 (planted in 2009, clone GG100) and CE (native cerrado vegetation), Distrito Federal, Brazil.

| Areas | Rainy season[1] 2014-2015 | Dry season[2] 2015 | Rainy season[1] 2015-2016 | Dry season[2] 2016 | Total year 1* | Total year 2* |
|---|---|---|---|---|---|---|
| | ---------- Litterfall (kg ha$^{-1}$) ---------- | | | | | |
| E1 | (415±105) aB | (584±184) aA | (270 ±121) aB | (324±118) bB | (6048 ±234) aA | (3579±104) bB |
| E2 | (441±122) aA | (507±137) aA | (405±106) aA | (384±98) bA | (5582±209) abA | (4845± 107) aA |
| CE | (314±98)aBC | (525±156) aA | (223±138) aC | (495±171) aB | (4076 ±277) bA | (4658±281)abA |

Different lowercase letters in a column differ between areas, different uppercase letters in a row differ between seasons (dry and rainy) and per year (Tukey, $p < 0.05$). [1]Rainy season (October to April) and [2]Dry season (May to September); Seasonal data = average yield per season; Total = sum of 12 yield months. *Year-1 (October 2014 to September 2015) and *year-2 (October 2015 to September 2016).

Regarding to the litter layer, for both years, the greatest stock of biomass in the forest litter was observed in the E2 (15 and 16 Mg ha$^{-1}$), followed by E1 (12 and 14 Mg ha$^{-1}$) and CE (7 and 8 Mg ha$^{-1}$), respectively (Figure 1.3). The stored carbon in this litter in the first year (October 2014 to September 2015) was 5.6 Mg ha$^{-1}$, 7.2 Mg ha-1, 3.6 Mg ha$^{-1}$, and in the second year (October 2015 to September 2016) was 7.2 Mg ha$^{-1}$, 7.8 Mg ha$^{-1}$ and 4.3 Mg

$ha^{-1}$ for the E1, E2 and CE areas, respectively (Figure 1.3). In the second year, the increase in biomass and carbon concentration, caused the increase of the litter carbon stock in E1 area ($F = 42.02$, $p < 0.01$).

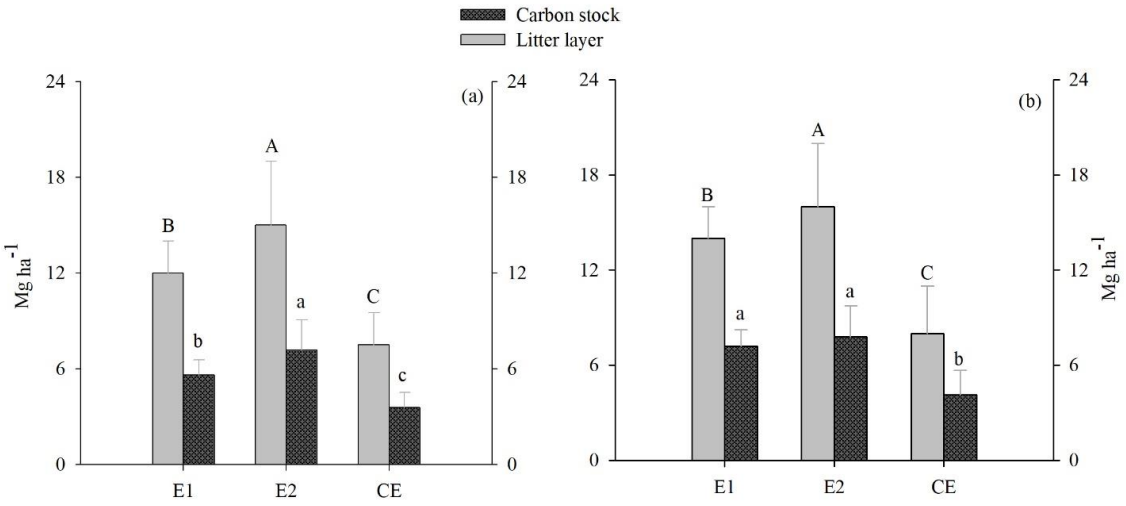


**Figure 1.3 -** Litter layer and Carbon stock year-1 (October 2014 to September 2015) (a) and year-2 (October 2015 to September 2016) (b) in *Eucalyptus* stands E1 (planted in 2011, clone EAC 1528) and E2 (planted in 2009, clone GG100) and CE (native cerrado vegetation), Distrito Federal, Brazil. Capital letter represent differences among litter layer and lowercase letters difference between Carbon stock ($p < 0.05$).

## 3.2. LITTER DECOMPOSITION

The litter decomposition constant (k) was 0.0013 for E1 and E2, and 0.0021 for the CE (Figure 1.4 a). The time required to reach 95 % mass loss ranged from 6.4 years in the E1 and E2 areas to 4 years in the CE, respectively, but lenghth of time to reach 99 % mass loss would be 4,1 and 2,6 extra years for Eucalyptus areas and CE. The three areas fitted to $R^2 \geq 0.97$ ($p < 0.01$). Litter decomposition was similar in the three areas until the 360$^{th}$ evaluation day. Thereafter, the loss of litter mass increased in the CE area, while no significant differences were observed in E1 and E2 (Figure 1.4b). The remaining litter mass in the areas was 54, 51, and 54 % on the 360$^{th}$ day and 35, 37 and 22 % on the 720$^{th}$ day for E1, E2 and CE, respectively (Figure 1.4b).

In the three areas, litter decomposition was positively correlated ($r \geq 0.80$, $p = 0.05$) with soil moisture. Therefore, litter mass loss was also correlated with soil moisture that was higher in the CE area (20 to 35 %; $F < 17.5$, $p = 0.008$), and ranged from 15 to 21 % in the areas E1 and E2 (Figure 1.4c) over the two-year assessment.

Litter mass loss at the end of the first and second year was 1.5, 1.6 and 1.5, and 2.2, 2.1 and 2.6 Mg $ha^{-1}$ for the E1, E2 and CE areas, respectively. This represents, on average, that the decomposition in the E1, E2 and CE areas was 7.9 %, 6.6 % and 16.8 % in relation to the litter layer stored above the ground.

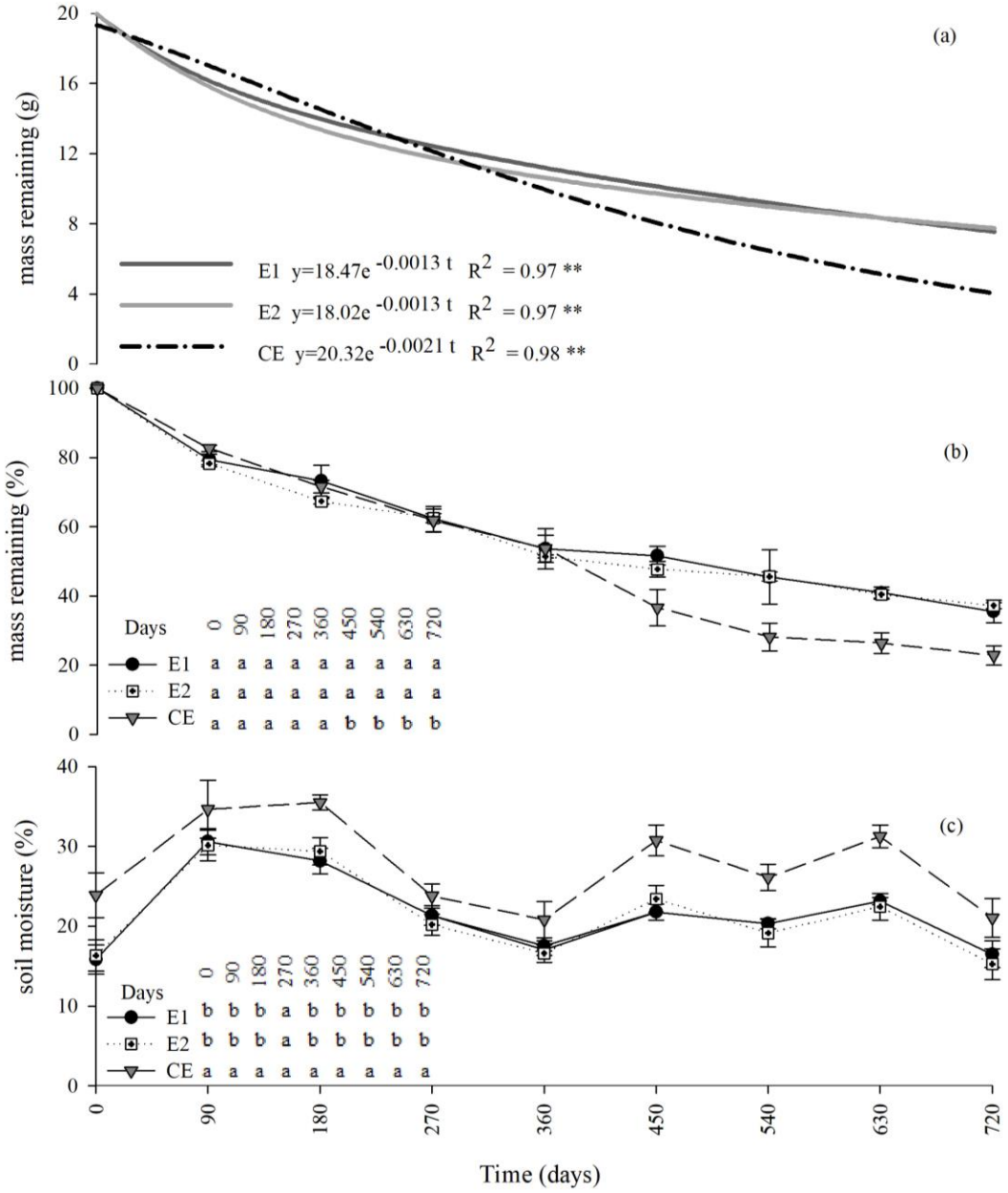


**Figure 1.4 -** Exponential curve and percentage of remaining litter mass (a), (b), soil moisture (c) over 720 days in *Eucalyptus* stands E1 (planted in 2011, clone EAC 1528) and E2 (planted in 2009, clone GG100) and CE (native cerrado vegetation), Distrito Federal, Brazil. Bars indicate standard deviations for each sampling day (n = 9) after time 0. Different letters indicate significant differences between areas for each sampling day after time 0 ($p < 0.05$).

3.3. LIGNIN, CELLULOSE AND HEMICELLULOSE CONTENTS

Throughout the 720 days of litter decomposition, the lignin contents were higher in CE on most days of the evaluation period ($p < 0.05$) (Figure 1.5a). In the same period, the lignin content increased from 11 % to 17 % in E1, from 11 % to 18 % in E2 and from 14 % to 29 % in CE.

Contrary to lignin, the cellulose contents decreased by 3 %, 5 % and 4 % in the areas E1, E2 and CE, respectively (Figure 1.5b). For the hemicellulose contents, statistical differences between the study areas were observed after 360, 450 and 540 days, with higher values in E2 than CE ($p < 0.05$). Throughout the evaluation period, the hemicellulose contents, unlike the other components, were constant (Figure 1.5c).

Correlations of -0.79 and -0.93 were observed between the lignin and remaining mass contents for E1 and E2, respectively, whereas for remaining mass and cellulose content, the correlations were > 0.75 in the studied areas.

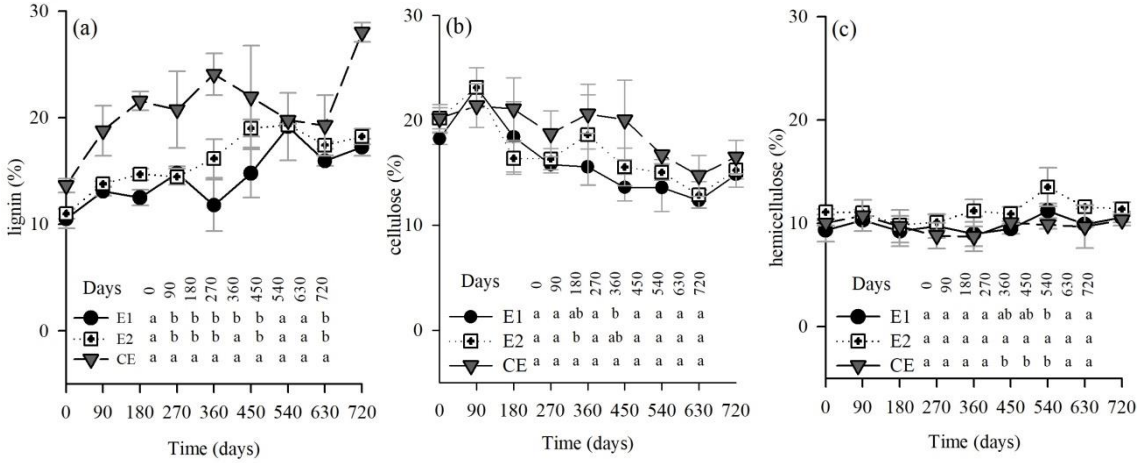


**Figure 1.5 -** Percentage of lignin (a), cellulose (b) and hemicellulose (c) of the remaining litter mass over a period of 720 days in *Eucalyptus* stands E1 (planted in 2011, clone EAC 1528) and E2 (planted in 2009, clone GG100) and CE (native cerrado vegetation), Distrito Federal, Brazil. Bars indicate the standard deviations for each sampling day (n = 9) after time 0. Different letters indicate significant differences between areas for each sampling day after time 0 ($p < 0.05$).

3.4. C, N AND P CONCENTRATIONS

A decreasing trend of the C concentration in the remaining litter mass was observed during the evaluation period. At the first sampling, the C concentrations were 530.5, 532.8

and 539.6 g $kg^{-1}$ and at the last, 474.8, 449.7 and 409.3 g $kg^{-1}$ for E1, E2 and CE, respectively (Figure 1.6a).

The N litter concentrations indicated an increase over time of decomposition, due to the mass loss in all evaluated areas. The N concentrations were statistically different at 450 days (Figure 1.6b, F = 11.53, p = 0.01) and at 630 days (Figure 1.6b, F = 10.59; p = 0.01). During 720 days the apparent liberation rate for N was 36 %, 33 %, 52 % in the E1, E2 and CE areas, respectively.

The remaining P was highest in E2 (p <0.007), except at time zero, compared to the other areas throughout the entire sampling period (Figure 1.6c). Although there was an increase in P concentration with increasing decomposition time of litter in the field, especially in area E2, which was higher in relation to the other areas. The apparent liberation rate for this element was 54 %, 38 %, 61 % in the E1, E2 and CE areas, respectively.

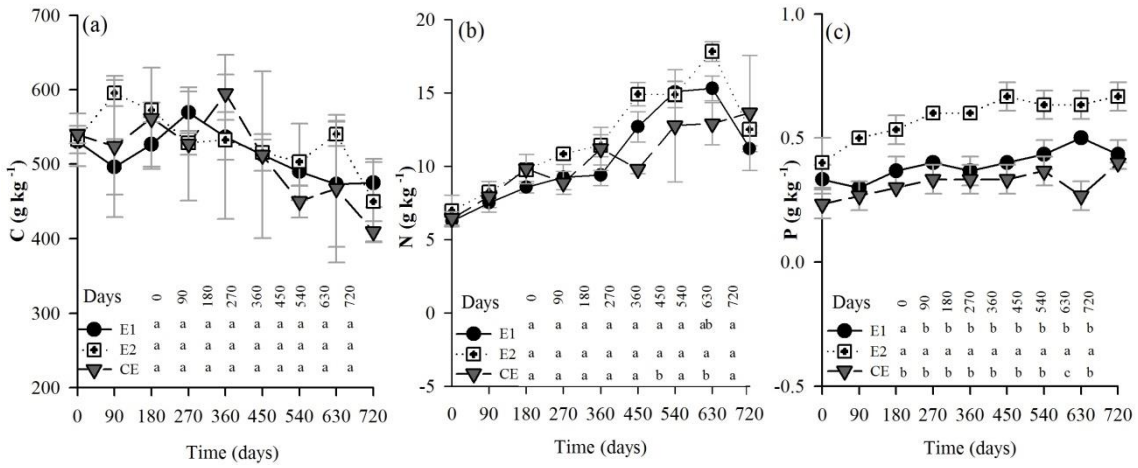


**Figure 1.6 -** Concentrations of C (a), N (b) and P (c) of the remaining litter mass during 720 days in *Eucalyptus* stands E1 (planted in 2011, clone EAC 1528) and E2 (planted in 2009, clone GG100) and CE (native cerrado vegetation), Distrito Federal, Brazil. Bars indicate the standard deviations for each sampling day (n = 9) after time 0. Different letters indicate significant differences between areas for each day of sampling after time 0 (p <0.005).

The C:N ratio tended to decrease in the evaluation period (720 days). In the initial phase (time 0) this ratio was C:N > 76:1 and at the end of the evaluation period C:N > 30:1. In the evaluations (n = 9), no significant differences were observed for the C:N ratio among the areas (Figure 1.7a), but differences in the C:N ratio at time 0 and after 720 days (E1 (F= 17.27; p < 0.01), E2 (F= 16.30; p < 0.01) and CE (F= 14.93; p < 0.001).

The C:P ratio also showed a decreasing pattern over time (Figure 1.7) and was lowest for E2 at most sampling times (Figures 1.7a and b). The correlations between remaining mass and C:N ($r > 0.92$, $p = 0.05$) and C:P ($r > 0.85$, $p = 0.005$) were positive and significant for all areas studied.

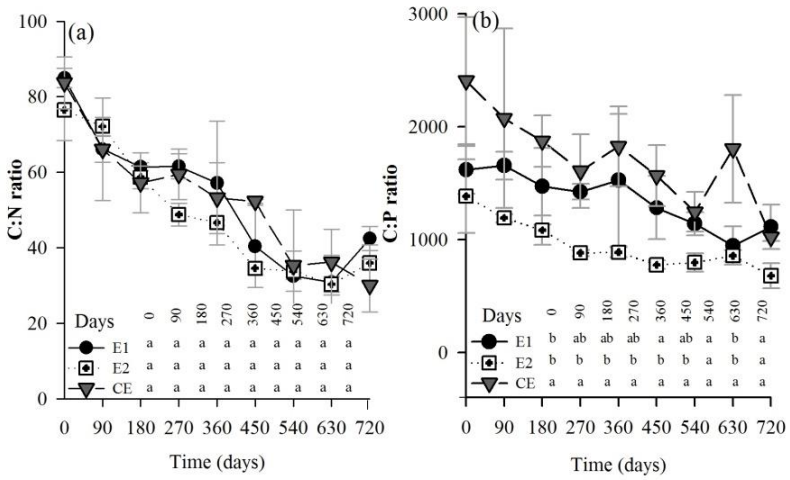


**Figure 1.7 -** Pattern of the C:N (a) and C:P (b) of the remaining litter mass over 720 days in *Eucalyptus* stands E1 (planted in 2011, clone EAC 1528) and E2 (planted in 2009, clone GG100) and CE (native cerrado vegetation), Distrito Federal, Brazil. Bars indicate the standard deviations for each sampling day (n = 9) after time 0. Different letters indicate significant differences between areas for each sampling day after time 0 ($p < 0.05$).

# 4. DISCUSSION

## 4.1. LITTERFALL AND LITTER LAYER DYNAMICS

The litter on the forest floor indicates a seasonal effect on litterfall (Table 1.1), since the native vegetation is adapted to the extreme rainfall seasonality of the Cerrado, by reducing plant transpiration during the dry season and by the presence of drought deciduous species that eventually shed their leaves. Several studies corroborate this finding (Giácomo et al., 2012; Souza et al., 2016; Oliveira et al., 2017) and also explain the greater input of litterfall and litter layer biomass in the dry season.

The largest litter layer in the E2 area, independent of the year, is due to the trees stand of E2 being older, with a denser canopy, resulting in more dropping of branches (natural pruning). After canopy closure, the leaves on branches at the canopy base are shaded, reducing the sunlight interception and intensifying natural pruning (Paiva and Leite, 2015).

In this trial, the litterfall in the *Eucalyptus* stands was lower than reported by other authors who studied different species of *Eucalyptus* (Schumacher et al., 2013; Viera et al., 2014; Inkotte et al., 2015). The litter layer, in turn, was higher (13.5 to 15 Mg $ha^{-1}$ $year^{-1}$) than found in other studies regarding *Eucalyptus* forests in Brasil (Gatto et al., 2014; Souza et al., 2016; Barbosa et al., 2017; Ribeiro et al., 2017; Santos et al., 2017).

In the case of planted forest systems, changes in litterfall and litter layer can be influenced by characteristics of the species, particularly of the chemical composition (Ferreira et al. 2016b), management, plant density (Hakamada et al., 2017), age of trees (Corrêa et al., 2016), type of clone (Conti-Junior et al., 2017) and climatic conditions (temperature and precipitation) (Binkley et al., 2017; Carvalho et al., 2017).

The results show that the litterfall do not always reflect the litter layer in the forest floor (Table 1.1 and Figure 1.3), notably in the E1 area, where greatest amount of litterfall in the first year, did not result in highest litter layer in the same year. The dynamics of the litter layer depends not only on the litterfall amount, but also on the combination of factors such as, decomposition rate, nutrient availability and forest age, that are decisive in the biogeochemical cycles and ecosystem structure (Schumacher et al., 2013; Guendehou et al., 2014; Pinto et al., 2016).

The cabon stock in the litter layer indicate increase during the studied period. Although the E2 presented larger carbon stocks in the litter layer, it was in the E1 that a higher rate of increase of C was observed from year to year (Figure 1.3). This behavior might have been affected by the forest growth stages of E1. Wink, et al. (2013), report that eucalyptus stands presented a significant variation of litter stock and carbon stock. The litter deposition and nutrient cycling in forest ecosystems are the main route of entry of C in the soil-plant system and increase rapidly with plantation age (Du et al., 2015).

## 4.2. LITTER DECOMPOSITION PROCESS

The litter decomposition rates are considered fast if there is small amounts of accumulation in the soil surface, a condition not found in the present study. For Olson (1963), this condition is reached when the values of k are between 1.0 and 4.0. The constants k found in this study were smaller than 1.0, indicating that all areas present a slow rate of decomposition.

The rapid decomposition within up to 90 days (Figure 1.4a) probably is results from the fragmentation of litter into smaller particles by physical agents, soil biota and the release of more soluble compounds such as sugars, starches and proteins, which are rapidly

consumed by decomposing organisms (Swift et al., 1979; Oliveira et al., 2016). After this period, most of the more resistant or recalcitrant structures, rich in lignin, as well as in leaf veins and petioles, remain, decreasing the decomposition rate over time (Hammel, 1997; Boer et al., 2005; Baumann et al., 2009).

The highest litter mass loss in the CE area can be explained by water availability and biodiversity. A greater diversity of native vegetation species (Table 3), provides a better environment and food supply for leaf-cutting ants (Silva et al., 2011), which leads to greater fragmentation of plant material, speeding up physical breakdown and biochemical transformation of organic material (Duxbury et al., 1989; Oliveira et al., 2017). Moreover, to keep leaf cutting ants population below the economic injury level, Brazilian *Eucalyptus* forest producers provide broadcast application of pesticides for long-term pest, especially during the first three years of plant age (Zanetti et al., 2014).

Another important finding in this study is the fact that native vegetation provided the highest water retention in the soil what might reflect in the litter moisture. Thus, higher soil moisture, favors maintenance of environmental conditions for preservation of litter food web (Nouvellon et al., 2012). Biological diversity of macro and meso fauna, microorganisms, as well as better microbiological properties, can be used as indicators of more fast decomposition (Oliveira et al., 2016). Castro et al. (2016) also report that the abundance of soil microbial communities is profoundly affected by the considerable seasonal variation in water availability, which is characteristic of the Cerrado biome.

The decomposition of the total biomass, and the cycling of most nutrients through litterfall and decomposition were at least twice higher than in the Cerrado *sensu stricto*. Thus, it is likely that the rapid and effective cycling of nutrients observed in the cerradão might be a key condition guaranteeing the ability of the cerradão to colonize new areas previously occupied by the typical Cerrado (Oliveira el al., 2017).

The greatest biomass stock in the litter layer of *Eucalyptus* stands resulted from the higher deposition and low decomposition of the plant material. In general, the genus *Eucalyptus* sp. has slow rates of litter decomposition, of generally less than 50 % within 12 months, independent of the management and soil-climate conditions (Adams and Attiwil 1986; Bachega et al., 2016; Souza et al., 2016). Other studies report a higher decomposition rate in relation to our findings, with a decomposition constant varying from 0.0015 to 0.56 (Schumacher et al., 2013; Cizungu et al., 2014; Viera et al., 2014; Pinto et al., 2016).

The chemical composition of Eucalypt leaves might decrease the microbial colonization, by the presence of essential oils and other allelopathic chemical compounds

that hinder the establishment and action of microorganisms, preventing colonization and subsequent decomposition and mineralization (Chu et al., 2014; He et al., 2014; Ferreira et al., 2016b).

## 4.3. COMPONENTS OF THE REMAINING LITTER

The increase in lignin content in the remaining litter mass after 720 days of evaluation in all three areas (Figure 1.5a) can be explained by the initial loss of more easily decomposed carbohydrates from the plant material. In addition, the decomposition rates are initially determined by rapidly proliferation bacteria, while in later stages, the rates are determined mostly by fungi (Swift et al., 1979). This behavior is a result of the increase in the proportion of more recalcitrant materials such as lignin (Boer et al., 2005; Baumann et al., 2009) throughout the decomposition process.

This organic fraction is described as the most resistant to degradation, which results in increasing concentration throughout the decomposition process, due to the release of the most soluble C forms initially (Hammel, 1997). It is worth mentioning that decomposition is a dynamic process, driven by a rapid succession of organism communities conditioned by the substrate quality and environmental conditions (Oliveira et al., 2016; Oliveira et al., 2017).

Litter decomposition was positively correlated with the reduction in contents of cellulose, the least recalcitrant of the analyzed carbon compounds. Although hemicellulose is the most labile structural component (Wagner and Wolf, 1999), cellulose was broken down more rapidly. There are indications that the release rate of hemicellulose is proportional to loss of mass, and the fact that its release is constant over time causes the hemicellulose not to be affected by the litter decomposition stage in two years of evaluation.

The N and P concentrations increased in the 720 days of evaluation, when the mass loss was positively correlated with nutrient concentration, indicating that the greater the loss, the higher the concentration of the elements in the remaining mass, suggesting immobilization of these nutrients throughout decomposition and C loss (Singh et al., 2004; Schumacher and Viera, 2015). During litter decomposition, there is an increase in the lignin content, which is a process that occurs throughout the study. In addition, an increase in N concentration is observed, which plays an important role in lignin degradation (Berg and Mcclaugherty, 2008).

The low variation in of N and P concentrations in the litter decomposition in CE can be attributed to low soil fertility and seasonality. Savanna species (Table 3) have ecological

strategies for the conservation of functional properties as well as nutrient maintenance and internal cycling, e.g., by nutrient retranslocation followed by leaf dehiscence (Lloyd et al., 2008; Vourlitis et al., 2013; Blaser et al., 2014), cycling nutrients with minimal losses.

The higher concentration of P in the E2 litter probably correlates with the residual effect of fertilization (Table 1). The soil of this area presented about three times more P when compared to the other forests E1 and CE (Table 2), reflecting in a higher concentration of this nutrient in the plant due to the history of use and soil management.

The C:N and C:P ratios had positive correlations with the litter mass loss, due to the C presenting an average release of 21 % for the three areas over the two-year evaluation. Nutrients and their relationship are complex by the fact that different organisms are involved in the decomposition process, these organisms may acquire nutrients from the abiotic environment in addition to those provided by the litter, and because the rates of decomposition are often limited by the lability of C compounds rather than by N or P availability (Gessner and Chauvet 1994).

## 5. CONCLUSIONS

In the Cerrado, litterfall and litter layer did differ between the *Eucalyptus* forest systems with different ages and clones. Seasonality influenced mainly the native vegetation, with higher biomass (litterfall) on the soil in the dry season, while in the rainy season, a higher mass loss was observed in all studied areas. Biomass and litter carbon can be influenced by age in the case of eucalyptus stands.

The litter decomposition is higher in the area of natural Cerrado vegetation, where soil moisture is more adequate. The lignin and cellulose contents function as indicators of litter decomposition in *Eucalyptus* and native plantations. The 720 days of evaluation were not enough to liberation the lignin and hemicellulose contents in any of the studied areas.

Apparently N and P liberation were more efficient in the native vegetation. It is also worth mentioning that if the study period had been only one year (360 days), no difference would have been detectable in the remaining litter mass between *Eucalyptus* stands and native vegetation. These findings reinforce the importance of long-term studies of native and planted forest ecosystems in the Cerrado.

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# CAPÍTULO 2

## $CH_4$ AND $N_2O$ FLUXES IN PLANTED FORESTS AND NATIVE ECOSYSTEMS IN THE BRAZILIAN CERRADO

**RESUMO**

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**Brasília, 23 de março de 2018**

Solos florestais são importantes fontes de $N_2O$ e comumente possuem comportamento de dreno em relação ao $CH_4$. Entretanto, é importante considerar a variabilidade temporal desses fluxos. Este estudo avaliou a dinâmica sazonal, bem como as interações com os condutores ambientais dos fluxos de $CH_4$ e $N_2O$ de solos sob plantios de eucaliptos e vegetação nativa do Cerrado. O estudo foi realizado no Distrito Federal, Brasil, durante um período de 26 meses, de outubro de 2013 a novembro de 2015, em três áreas: plantações de eucaliptos híbridos (*Eucalyptus urophylla* x *Eucalyptus grandis*) implantados em 2011 (E1) e em 2009 (E2) e vegetação nativa do Cerrado (CE). As amostras de ar foram coletadas usando uma câmara de fechamento manual e a concentração de gás foi determinada por cromatografia gasosa. A temperatura do ar e do solo, o espaço poroso saturado por água (EPSA) e as concentrações de nitrogênio mineral, nitrato ($NO_3^-$) e amônio ($NH_4^+$) também foram monitorados. O comportamento dos fluxos diários de gases foi analisado para cada estação (chuvosa e seca) e ano (total acumulado anual). Não houve um padrão claro em resposta às variações sazonais entre as áreas. Para as áreas E1, E2 e CE, respectivamente, os fluxos médios de $CH_4$ foram -35, -3 e -2 μg de C-$CH_4$ $m^{-2}$ $h^{-1}$ no primeiro ano (ano 1) e -22, -8 e -1 μg C-$CH_4$ $m^{-2}$ $h^{-1}$ no segundo (ano 2). Quanto à sazonalidade, os fluxos de $CH_4$ mais elevados de CE foram observados na estação chuvosa (11 μg C-$CH_4$ $m^{-2}$ $h^{-1}$) no ano 1, enquanto que no ano 2 foram estatisticamente iguais nas três áreas. Na estação seca, os fluxos foram mais elevados em E2 (9 μg C-$CH_4$ $m^{-2}$ $h^{-1}$) no ano 1 e, novamente, estatisticamente iguais no ano 2 entre as demais áreas. No primeiro ano, os fluxos médios de $N_2O$ foram 4, 8 e 3 μg de N-$N_2O$ $m^{-2}$ $h^{-1}$ e no segundo 5, 5 e 4 μg de N-$N_2O$ $m^{-2}$ $h^{-1}$ em E1, E2 e CE, respectivamente. Na estação chuvosa, os fluxos de $N_2O$ foram maiores em E2 (4 μg N-$N_2O$ $m^{-2}$ $h^{-1}$) no ano 1, mas maior em E1 no ano 2 (7 μg N-$N_2O$ $m^{-2}$ $h^{-1}$). Ao longo do período de avaliação, os influxos acumulados de $CH_4$ foram de -1,86 a -0,63 kg $ha^{-1}$ $ano^{-1}$ (ano 1) e de -1,85 a -1,34 kg $ha^{-1}$ $ano^{-1}$ (ano 2). Os fluxos cumulativos de $N_2O$ nas três áreas foram ≤ 0,85 kg $ha^{-1}$ $ano^{-1}$ no ano 1 e ≤ 0,44 kg $ha^{-1}$ $ano^{-1}$ no ano 2. Esta avaliação também sugeriu que os picos de fluxos de $CH_4$ e $N_2O$ ocorrem apenas alguns dias por ano e, portanto, têm pouco impacto nos fluxos anuais totais. A análise de todo o período de estudo indicou a captação de $CH_4$ da atmosfera e a contribuição do $CH_4$ e $N_2O$ para o potencial de aquecimento global (PAG) variou de 82 a 228 Kg $CO_2$ eq $ha^{-1}$ $ano^{-1}$ para os plantios de eucalipto e de 57 a 82 Kg $CO_2$ eq $ha^{-1}$ $ano^{-1}$ e vegetação nativa do Cerrado.

**Palavras-chave:** Gases de efeito estufa, mudança do uso da terra, savana.

**ABSTRACT**

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Forest soils are $N_2O$ sources and commonly act as $CH_4$ sinks. However, the temporal variability of these fluxes must be taken into account. This study evaluated seasonal dynamics as well as interfaces with environmental drivers of $CH_4$ and $N_2O$ fluxes from soils under *Eucalyptus* stands and native vegetation of Cerrado. The study was carried out in Distrito Federal, Brazil, over a 26 months period from October 2013 to November 2015, in three areas: hybrids eucalyptus plantations (*Eucalyptus urophylla* x *Eucalyptus grandis*) planted in 2011 (E1) and in 2009 (E2), and native Cerrado vegetation (CE). Air samples were collected using manual close chamber and gas concentration was determined by gas chromatography. Air and soil temperature, water-filled pore space (WFPS), and concentrations of mineral nitrogen, nitrate ($NO_3^-$), and ammonium ($NH_4^+$) were also monitored. The behavior of the daily gas fluxes was analyzed for each season (rainy and dry) and year (annual cumulative total). There was no clear pattern in response to seasonal variations among areas. For the areas E1, E2 and CE, respectively, the mean $CH_4$ fluxes were -35, -3 and -2 μg C- $CH_4$ $m^{-2}$ $h^{-1}$ in the first year (year 1) and -22, -8 and -1 μg C-$CH_4$ $m^{-2}$ $h^{-1}$ in the second (year 2). In terms of seasonality, highest $CH_4$ fluxes from CE were observed in the rainy season (11 μg C-$CH_4$ $m^{-2}$ $h^{-1}$) in year-1, while in year-2, they were statistically equal in the three areas. In the dry season, the fluxes were highest in E2, (9 μg C-$CH_4$ $m^{-2}$ $h^{-1}$) in year 1 and statistically equal again in year-2 for the other areas. In the first year, the mean $N_2O$ fluxes were 4, 8 and 3 μg N-$N_2O$ $m^{-2}$ $h^{-1}$ and in the second 5, 5 and 4 μg N-$N_2O$ $m^{-2}$ $h^{-1}$ in E1, E2 and CE, respectively. In the rainy season, $N_2O$ fluxes were highest in E2 (4 μg N-$N_2O$ $m^{-2}$ $h^{-1}$) in year 1, but highest in E1 in year 2 (7 μg N-$N_2O$ $m^{-2}$ $h^{-1}$). Throughout the evaluation period, cumulative $CH_4$ influxes were -1.86 to -0.63 kg $ha^{-1}yr^{-1}$ (year-1) and -1.85 to -1.34 kg $ha^{-1}yr^{-1}$ (year-2). The cumulative $N_2O$ fluxes in the three areas were ≤0.85 kg $ha^{-1}yr$-$^{1}$ in year 1 and ≤0.44 kg $ha^{-1}yr^{-1}$ in year 2. This evaluation also suggested that high $CH_4$ and $N_2O$ pulses occur on only a few days a year and therefore have little impact on the total annual fluxes. The analysis of the entire study period indicated $CH_4$ uptake from the atmosphere and the contribution of $CH_4$ and $N_2O$ to global warming potential (GWP) ranged from 82 to 254 Kg $CO_2$ eq $ha^{-1}$ $yr^{-1}$ for eucalyptus plantations and from 83 to 98 Kg $CO_2$ eq $ha^{-1}$ $yr^{-1}$ in the native vegetation of Cerrado.

**Key words:** Greenhouse gases, eucalyptus, land-use change, savanna.

# 1. INTRODUCTION

Forests play a key role in the C cycle, by absorbing $CO_2$ (Martins et al., 2015a), as well as minimizing global warming by contributing to mitigate greenhouse gases (GHGs), including nitrous oxide ($N_2O$) and methane ($CH_4$) (Castaldi et al., 2013; Martins et al., 2015a; Santos et al., 2016) However, few studies have addressed $N_2O$ and $CH_4$ fluxes from tropical savanna systems, which raises the level of uncertainty with regard to the role of these ecosystems in GHG emissions (Castaldi et al., 2013; IPCC, 2013; Aini et al., 2015).

As a result of anthropic activity, an augmented abundance of GHGs in the atmosphere has been observed since the Industrial Revolution, which is largely responsible for the increases recorded in global temperatures (IPCC, 2013; WMO, 2016). However, climate projections further increase uncertainties in future GHG measurements. In 2010, GHG emissions were estimated at 49 x $10^9$ Mg $CO_2$eq. (IPCC, 2014), of which 21-24 % represented soil uses for agriculture, forestry and other land uses (Tubiello et al., 2013; IPCC, 2014).

In terms of $CO_2$eq, the atmosphere reached a concentration of 485 ppm in 2015 (WMO, 2016). Considering the contributions of $CH_4$ and $N_2O$, the annual emission rates increased faster from 2014 to 2015 (11.5 ppb $yr^{-1}$) than in the period from 2007 to 2013 (5.7 ± 1.2 $ppb^{-1}$) (Butler and Montzka 2016), while $N_2O$ in the atmosphere increased 0.18 % between 2015 and 2016, i.e., a continuous mean increase of 0.60 ppb yr-$^1$ (WMO, 2016). In 2015, Brazil issued about 1.927 billion gross tons of $CO_2$eq, an increase of 3.5 % compared to 2014 (SEEG, 2016).

Soils are important sources and sinks of these GHGs (Spahni et al., 2011). Approximately 70 % and 35 % of the total $N_2O$ and $CH_4$ emitted into the atmosphere are from the soil, but with lower concentrations than $CO_2$, although the warming potential of the two gases was 298 and 25 times higher than that of $CO_2$, respectively, considering a period of 100 years (IPCC, 2007).

The interpretation of the GHG fluxes, particularly those of $CH_4$ and $N_2O$, is complex, due to the influence of soil-climate processes and also to the synergism of factors, resulting in high temporal and spatial variability (Kim et al., 2016). However, the forest type (Masaka et al., 2014) and soil management practices can be relevant in the control of GHG fluxes (Kim et al., 2016), as well as land use changes (Kim and Kirschbaum, 2015), Water-

filled pores space (Santos et al., 2016) and N input (Carvalho et al., 2017; Hickman et al., 2015).

With regard to $CH_4$, ecosystems differ significantly. Seasonally dry forests and savannas are usually sinks (Ciais et al., 2013; Valentini et al., 2014), due to the good conditions of drainage and aeration that favor $CH_4$ oxidation by methanotrophic bacteria (Liu et al., 2009). The factors driving this oxidation are gas diffusivity and soil temperature (Castaldi et al., 2013) and soil water content (Santos et al., 2016).

For $N_2O$, the N soil availability is one of the key regulators of fluxes. However, recent research indicates that, in the absence of increases in N input into the system, the native Cerrado vegetation may be a biome that naturally mitigates $N_2O$ emissions (Martins et al. 2015a; Santos et al., 2016; Carvalho et al., 2017; Sato et al., 2017) and $N_2O$ fluxes can be found even below the detection limit (Bustamante et al., 2012).

Native forests and commercial *Eucalyptus* stands of different ages have been little studied with regard to the potential emission or consumption of GHGs in the Cerrado region, but are main drivers for the fluxes of these GHGs. Consequently, this study aimed to reduce regional uncertainties by contributing with a two-year monitoring program of annual and seasonal effects on GHGs in different forest environments. Therefore, the objectives of this study were: (a) to evaluate the $CH_4$ and $N_2O$ fluxes of soils under planted *Eucalyptus* forests and native Cerrado vegetation, (b) describe the fluxes and their interactions with seasonality and environmental variables, and (c) to determine the cumulative and the Global Warming Potential (GWP) the $N_2O$ and $CH_4$ fluxes in soils of planted *Eucalyptus* forests of different ages and native Cerrado vegetation.

## 2. MATERIAL AND METHODS

### 2.1. STUDY SITE

See the description in general methodology (page 4).

### 2.2. MEASUREMENTS OF $CH_4$ AND $N_2O$ FLUXES

The $CH_4$ and $N_2O$ fluxes were measured from October 2013 to November 2015, separated in year-1 (October 2013 to September 2014) and year-2 (October 2014 to November 2015). Years were kept separate towards investigating effects of changes both in

the age of eucalyptus trees, and climate variables, specially annual and monthly rainfall, on GHG fluxes, that otherwise would go unnoticed on whole of data set.

The closed-chamber method was used for the measurements (Alves et al., 2012), in a sampling frequency of three times per month. Three 30 m x 30 m plots were randomly delimited in each area. Four closed chambers were installed per plot, two in the *Eucalyptus* rows and two in-between the rows, within a distance of about 10 m away from each other. In CE, the four manual gas chambers were placed randomly in each plot, resulting in a total of 36 chambers installed in the areas.

Each closed chamber consisted of a metal base (0.38 m x 0.58 m) inserted in the soil to a depth of 5 cm, sealed with a top part made of a PVC tray quilted with a 9.5 cm thick aluminized thermal blanket in order to ensure no gas leakage.

A hole was drilled in the center of the top part of each chamber and connected with a rubber hose and a three-way valve, by which the gas outlet could be controlled at sampling. Digital thermometers were installed to monitor the air temperature within the chambers.
The soil temperature at 5 cm depth was measured with a digital thermometer (model Incoterm®) at the sampling times. The gas samples were measured always in the morning, between 09:00 and 11:00 a.m, representing the daily mean emission conditions (Alves et al. 2012).

The air trapped in the chambers were sampled at 0, 15 and 30 min after closing the device. A 60 mL polypropylene syringe was used, coupled with a three-way valve, in which 30 mL gas samples were collected and transferred to evacuated vials. In addition, one sample of atmospheric gas per plot was taken, as reference for the analysis of the gas samples. Before and after sampling, the vials were transported in ice-cooled thermal boxes and then stored in a refrigerated environment at 16 ºC for measurements.

The $CH_4$ and $N_2O$ concentrations were determined by Gas Chromatography (Trace 1310 GC ultra, Thermo Scientific ™) equipped with a Porapak Q column at 65 °C, an electron capture detector (ECD) and flame ionization detector (FID). The following standards were used: 200 ppb, 600 ppb, 1000 ppb, and 1500 ppb $N_2O$; and 1000 ppb, 5000 ppb, 10000 ppb, and 50000 ppb $CH_4$. Based on the calibration curve, the calculated detection limit was 55 ppb for $N_2O$ and 145 ppb for $CH_4$ and the calculated quantification limit was 154 ppb for $N_2O$ and 484 ppb for $CH_4$. The $CH_4$ and $N_2O$ fluxes were measured by the linear variation in gas concentration in relation to the incubation time in the closed chambers, and calculated by Equation (1), as proposed by Bayer et al. (2015):

$$Flux = \delta C/\delta t\ (V/A)\ m/Vm \quad (1)$$

Where the flux (μg $m^{-2}$ $h^{-1}$); δC/δt is the change in gas concentration (nmol $N_2O$ and $CH_4$ $h^{-1}$) in the chamber in the incubation interval (t); V and A are, respectively, the chamber volume (V) and the soil area covered by the chamber ($m^2$); m is the molecular weight of $N_2O$ and $CH_4$ (μg), and Vm is the molar volume at the sampling temperature (Vm).

The fluxes were calculated separately for the sampling times 0, 15 and 30 min, obtained in μg $N_2O$-N $m^{-2}$ $h^{-1}$ and μg $CH_4$-C $m^{-2}$ $h^{-1}$. The mean daily $N_2O$ and $CH_4$ fluxes were calculated from the mean value of the four chambers installed per plot. To determine the cumulative fluxes, the area under the curve was integrated based on the daily $N_2O$ and $CH_4$ soil fluxes (Santos et al., 2016). The amount of equivalent carbon (C eq) required to mitigate the cumulative annual fluxes of $CH_4$ (C-$CH_4$) and $N_2O$ (N-$N_2O$) was calculated by Equations 2 and 3, respectively.

$$C\text{-}CH_4 = (CAI^* 16/12) * GWP * F \quad (2)$$

$$N\text{-}N_2O = (CAF^* 44/28) * GWP * F \quad (3)$$

Where: CAI - Cumulative annual influx (kg C-$CH_4$ $ha^{-1}$ $yr^{-1}$); GWP - global warming potential of $CH_4$ (25 kg $CO_2$, IPCC (2007); F - factor 0.273 (used for the conversion from $CO_2$ to C); CAF - Cumulative annual flux (kg N-$N_2O$ $ha^{-1}$ $yr^{-1}$); GWP - global warming potential of $N_2O$ (298 kg $CO_2$, IPCC (2007); F - factor 0.273 (used for the conversion from $CO_2$ to C).

The global warming potential of emission GWP (over a 100 year horizon) expressed in $CO_2$eq was calculated by multiplying the accumulated emissions of each gas by its radiative forcing. For this, the conversion factor of 25 and 298 kg $CO_2$ $kg^{-1}$ gas (IPCC, 2007) was used, as follows in equation 4:

$$\text{GWP} = (CH_4\ x\ 25) + (N_2O\ x\ 298) \quad (4)$$

where: GWP is the Global Warming Potential (kg $CO_2$ eq $ha^{-1}$ year $^{-1}$), $N_2O$ and $CH_4$ correspond to the accumulated emissions of each gas (kg $ha^{-1}$) and the conversion factors used were 25 for $CH_4$ and 298 for $N_2O$.

2.3. ENVIRONMENTAL VARIABLES

The precipitation in Year 1 was 1210.9 mm, with 98% of the rain concentrated in the rainy season. While second year, total precipitation was 1305.6 mm, of which 95% was concentrated in the rainy season, the mean air temperature was 21.4 and 22.2 °C for year 1 and 2, respectively (Figure 2.1).

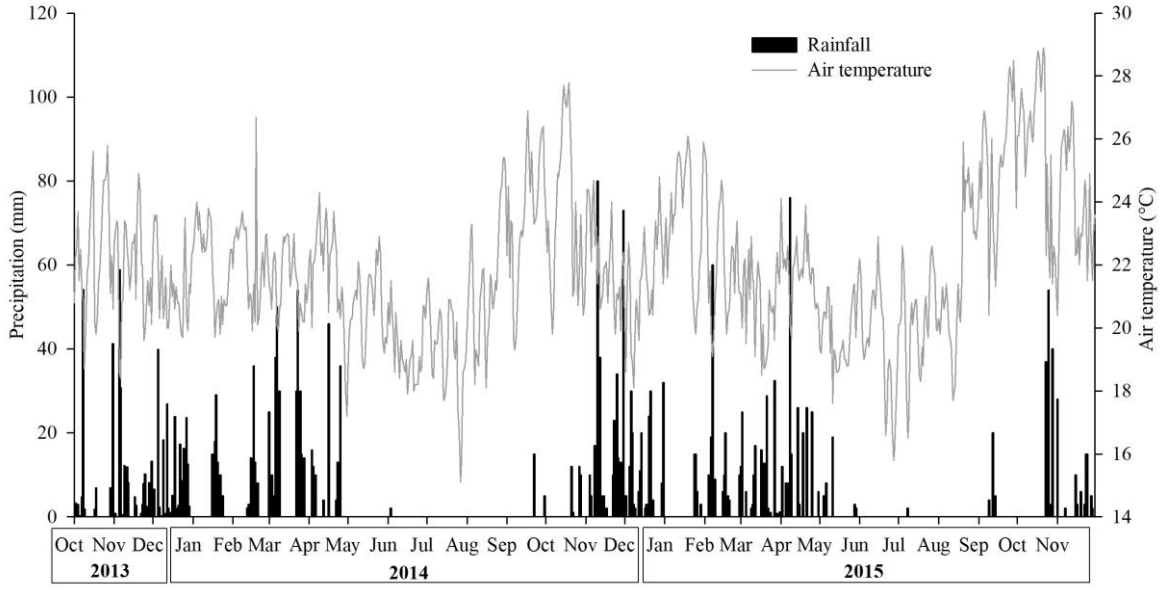


**Figure 2.1-** Precipitation (mm) and air temperature (ºC) from October 2013 to November 2015, Distrito Federal, Brazil.

For each gas sample event, soil samples were also collected from the 0-5 cm layer for the determination of mineral nitrogen in the forms of nitrate ($NO_3^-$) and ammonium ($NH_4^+$), at eight points near the chambers, to form a composite sample. From each soil sample a sub-sample was taken to determine gravimetric soil moisture. For the extraction of $NO_3^-$ and $NH_4^+$, 50 mL of the 2 mol $L^{-1}$ KCl solution were used according to the methodology of Bremner and Mulvaney (1982) and analyzed by distillation by the Kjeldahl method (from May/2014 to January/2015) and from February 2015 onwards by the spectrophotometry method with a system of flux injection analysis (FIA) (Hambridge, 2007 a, b) to determine the $NO_3^-$ and $NH_4^+$ concentrations.

Soil particle density was determined by the ring and volumetric flask methods, respectively (Embrapa, 1997). Soil moisture was calculated by oven-drying a soil sub-sample of known weight at 105 ºC for 48 hours. From these variables, the water-filled pore space (WFPS) was calculated for each gas sampling date and determined by equation 5:

$$\text{WFPS} = (\theta x\ Da)/\ [1 - (Da/Dp)]\ x\ 100 \quad (5)$$

where θ is the soil moisture (g $g^{-1}$); Da the apparent soil density (g $cm^{-3}$); and Dp the soil particle density (2.65 g $cm^{-3}$).

The meteorological data were recorded at an automatic weather station (Campbell Scientific CR 1000) installed near the study area.

## 2.4. FOREST LITTER SAMPLING AND ANALYSIS

The chemical characterization of the forest litter was based on litter material, collected in a metallic frame, 50 cm x 50 cm, from which eight sub-samples with three replicates were collected per area. Soil material in contact with the litter layer was also collected from a depth of 2 cm to determine mineral N ($NO_3^-$ and $NH_4^+$) by the Kjeldahl method.

In the litter samples, dry matter and crude lignin were determined by the sequential method adapted from Campos et al. (2004). Total C and N were determined with an elemental analyzer CHNS (Vario Elemental Analyzer MACRO Cube CHNS - Elementar).

## 2.5. CALCULATIONS AND STATISTICAL ANALYSIS

The environmental variables were subjected to descriptive statistical analysis and applied to the normality test (Shapiro-Wilk) followed by analysis of variance (ANOVA). The daily $CH_4$ and $N_2O$ fluxes had a non-normal distribution, so a nonparametric Kruskal-Wallis test of medians was performed at 5 % probability, to find possible differences among the areas and the years studied by comparisons.

The $CH_4$ influxes and accumulated $N_2O$ fluxes for the sampling dates were calculated by linear interpolation. To compare areas and years, the data of accumulated fluxes and equivalent carbon were subjected to analysis of variance (ANOVA) and the Tukey test ($p < 0.05$).

The data of $CH_4$, $N_2O$ and of the variables (WFPS, soil temperature, $NO_3^-$ and $NH_4^{+)}$ were also subjected to multivariate analysis (principal component analysis - PCA) to check the variation and annual behavior in the studied period, resulting in the establishment of a diagram of the order of variables. Statistical analyses were performed with the Statistical Analysis System, version 9.1.3 (SAS Institute Inc., Cary, NC, USA).

Another analysis was the evaluation by the Pearson correlation of the effect of seasonality and of the relationship between $CH_4$ and $N_2O$ fluxes and the environmental

variables, using the FactoMineR free program R (version 3.2.2). Only significant correlations are shown in the text.

The litter-related variables (litter stock, carbon, nitrogen and lignin) were compared by the Tukey's Studentized Range (HSD) test ($p < 0.05$). Additionally, a paired t-test for $NO_3^-$ and $NH_4^+$ was carried out to compare the mineral N in the surface layer (0-2 cm deep), in the rainy and dry seasons.

## 3. RESULTS

### 3.1. LITTER QUALITY IN FOREST SOILS

The litter layer varied according to the areas, without significant statistical differences between the different ages of planted forests stands. Even though the litter varied with regard to litter layer, this did not affect the concentrations of C (~ 46 %) and N in all areas (Table 2.1). The C:N ratio in the litter ranged from 66:1 to 75:1and the lignin content was highest in the CE area ($p<0.0054$).

**Table 2.1 -** Litter layer components in *Eucalyptus* stands E1 (planted in 2011, clone EAC 1528), E2 (planted in 2009, clone GG100); and CE (vegetation native to the cerrado), Distrito Federal, Brazil.

| Areas | Ll | C | N | L | C:N |
|---|---|---|---|---|---|
| | Mg $ha^{-1}$ | ---------- g $kg^{-1}$ ---------- | | | - |
| E1 | 10,78 ab | 463,4a | 6,15a | 99,5b | 75:1 |
| E2 | 13,7a | 465,7a | 7,04a | 114,6b | 66:1 |
| CE | 7,61b | 460,0a | 6,53a | 162,0a | 70:1 |

Ll= litter layer; C = carbon; N = nitrogen L = lignin. Different lowercase letters indicate significant differences between areas by the Tukey HSD test at 5 % probability.

### 3.2. TEMPORAL VARIATION IN $CH_4$ AND $N_2O$ FLUXES AND ENVIRONMENTAL FACTORS

The daily and seasonal $CH_4$ flows for year-1 and year-2 in each area are presented in Figure 2.2a. The mean annual $CH_4$ fluxes were -35, -3 and -2 μg $m^{-2}$ $h^{-1}$ for the year-1 and -22, -8 and -1 μg $m^{-2}$ $h^{-1}$ for the year-2 in E1, E2 and CE, respectively. Regarding the effect of seasonality significant differences among areas were observed in year-1, period during which median $CH_4$ flux in CE was higher than in the other two areas (p-value < 0.0196) in

the rainy season; nevertheless, in the dry season, the median $CH_4$ fluxes were highest in E2 (p-value = 0.0191).

The mean annual $N_2O$ fluxes were 4, 8 and 3 μg $m^{-2}$ $h^{-1}$ for year-1 and 5, 5 and 4 μg $m^{-2}$ $h^{-1}$ for year-2 in E1, E2 and CE, respectively (Figure 2.2b). In year-1 for $N_2O$ fluxes, significant differences were observed in the rainy season, with highest median $N_2O$ fluxes in E2 (p-value = 0.0196). While in the year-2, a significant difference was observed only in the rainy season, between the median fluxes in E1 and CE (p-value = 0.0273), which was higher for E1.

In the total evaluated period of GHGs, the CE presented the highest frequency of positive values. Positive $CH_4$ fluxes in the CE represented in the mean 44 % of the measurements, while in the *Eucalyptus* stands, this percentage was 19 %. With regard to seasonality, in year-1, the highest $CH_4$ fluxes were found in the rainy season for CE and in the dry season for E2. For the same year the highest $N_2O$ fluxes in the rainy season were noted in E2. In year-2, the highest $N_2O$ fluxes were observed in E1. No differences in seasonality were observed for the $CH_4$ and $N_2O$ fluxes within each area.

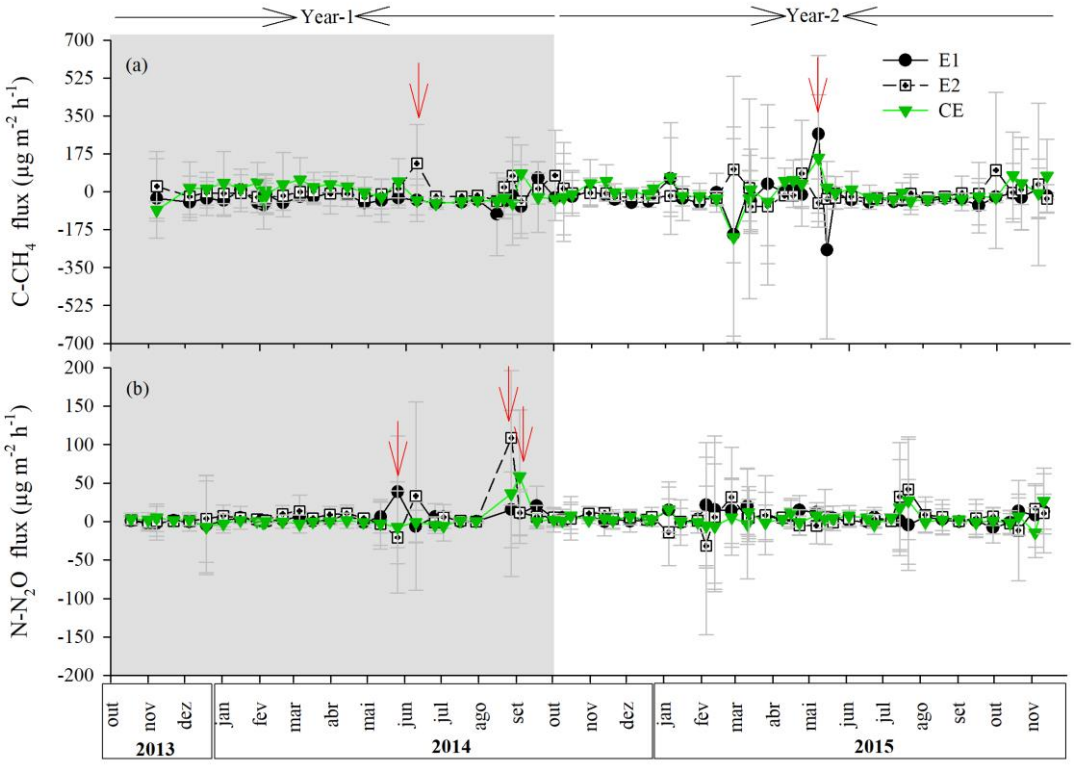


**Figure 2.2 -** Soil fluxes of methane - C-$CH_4$ (a), nitrous oxide - N-$N_2O$ (b), in *Eucalyptus* stands E1 (planted in 2011, clone EAC 1528) and E2 (planted in 2009, clone GG100); and CE (native cerrado vegetation), Distrito Federal, Brazil. Bars represent the standard deviation.

In relation to the environmental variables, it was observed that the $CH_4$ emission peaks (266, 131 and 156 μg $m^{-2}$ $h^{-1}$) coincided with WFPS values of 25 % for E1, 47 % for E2 and 68 % for CE (Figures 2.2a and 2.3a). The $N_2O$ peaks were (39, 108 and 58 μg $m^{-2}$ $h^{-1}$) with WFPS values varying from 23 % to 68 % (Figures 2.2b and 2.3a)

In this study, WFPS ranged from 9 % to 73 % in the rainy season and from 25 % to 46 % in the dry season. The Cerrado was the only area with a correlation of 0.58 (p-value = 0.0018) and -0.68 (p-value = 0.0015) between $CH_4$ flux and WFPS, respectively, in the rainy and dry season. Only in E2 correlations were detected between $N_2O$ fluxes and WFPS in the rainy and dry seasons (0.63 (p-value = 0.0005) and -0.59 (p-value = 0.0041), respectively). In year-2, WFPS ranged from 19 % to 71 % in the rainy season and from 30 % to 43 % in the dry season. With respect to correlations, a positive one was detected in CE between $CH_4$ and WFPS of 0.84 (p-value <0.0001). For $N_2O$, a correlation with WFPS of 0.62 (p-value = 0.0190) was observed in E1, both correlations occurred in the dry season.

The highest soil $NO_3^-$ and $NH_4^+$ concentrations were observed in the rainy season in all areas (p-value <0.0240) (Figures 2.3b and c) and did not coincide with the GHG emission peaks. However, in the rainy season, a correlation of -0.68 between $CH_4$ x $NO_3^-$ (p-value = 0.0004) in E1 was identified; of 0.49 between $N_2O$ x $NO_3^-$ (p-value = 0.0033) in CE; and 0.53 between $N_2O$ x $NH_4^+$ (p-value = 0.0023) in CE. In the dry season, the correlation was 0.55 between $CH_4$ x $NO_3^-$ (p-value = 0.0065) in CE.

The ammoniacal form of N was predominant, the $NO_3^-$ in the soil had highest concentrations in the rainy season (p-value <0.0093) and the $N_2O$ peaks coincided with $NO_3^-$ concentrations lower than 3.0 mg $kg^{-1}$ of soil (Figure 2.3b). The seasonality effect on $NH_4^+$ concentrations differed only in the CE area, with highest soil concentrations in the dry season (p-value <0.0001), coinciding with the $N_2O$ pulses in all the studied areas. In year-2, the $NO_3^-$ and $NH_4^+$ soil concentrations were very close between areas except for E2, where concentrations were below 1 mg $kg^{-1}$ of soil, from February 2015 onwards (Figures 2.3b e c). As for the correlations, E1 was the only area with negative correlation -0.63 (p-value = 0.002) between $N_2O$ flux and $NO_3^-$ content in soil.

In year-1, the soil temperature in all areas was of 17-24 ºC. The highest $CH_4$ and $N_2O$ pulse occurred with soil temperature ≥ 19 ºC, and correlation with $CH_4$ of 0.65 (p-value = 0.023) (Fig. 3d). In year-2, the soil temperature ranged from 16-26 ºC and correlated with CH4 flux in E2 (0.59; p-value = 0.024).

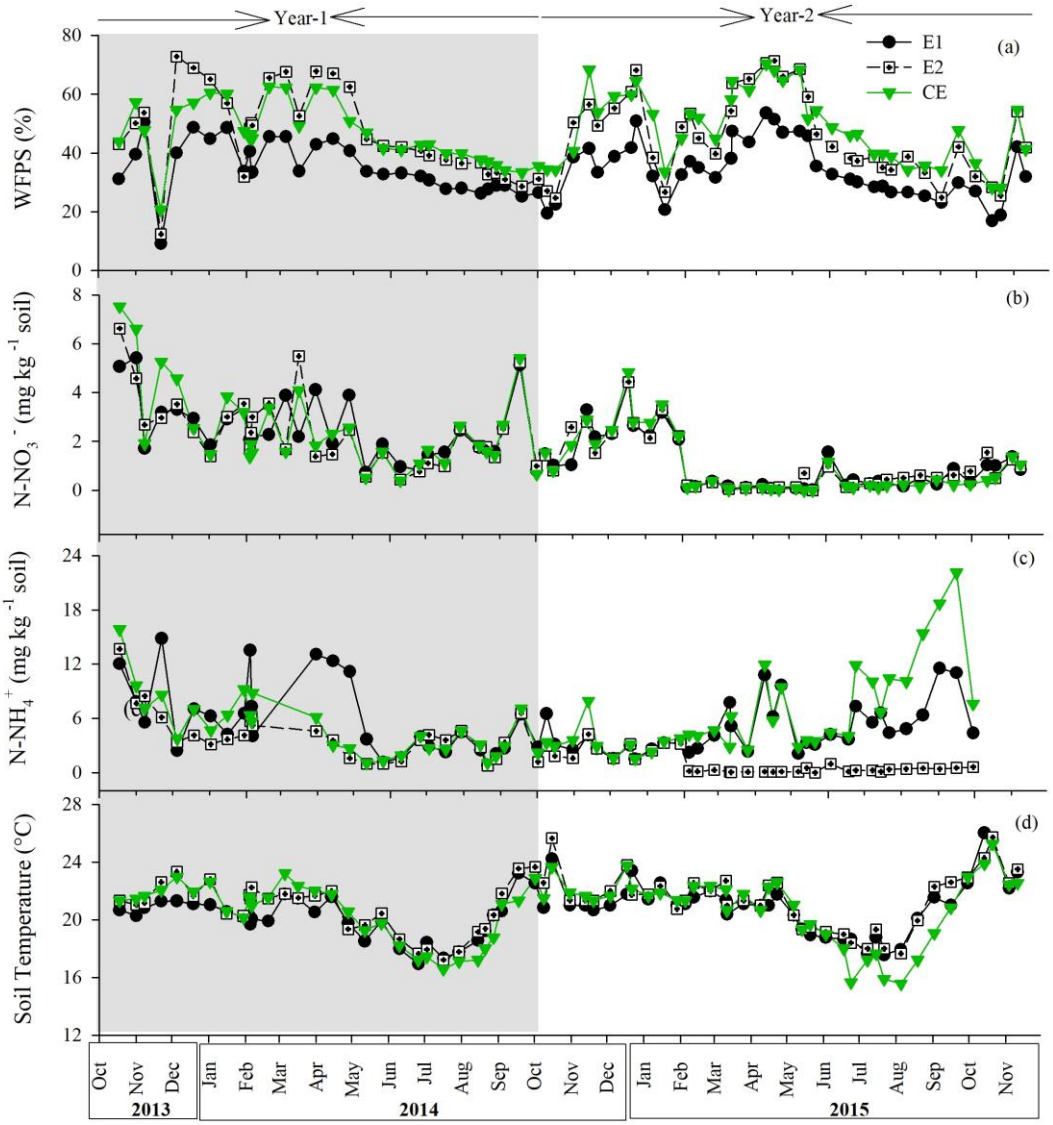


**Figure 2.3 -** Water-filled porous space - WFPS (a), nitrate $NO_3^-$ (b) ammonium $NH_4^+$ (c), and soil temperature (d) from October 2013 to November 2015 in Eucalyptus stands E1 (planted in 2011, clone EAC 1528) and E2 (planted in 2009, clone GG100); and CE (native cerrado vegetation), Distrito Federal, Brazil.

In relation to the mineral N form in the soil surface layer and in contact with the litter (0-2 cm deep), $NH_4^+$ was also predominant and had a differentiated behavior in the studied periods. In the rainy season, $NO_3^-$ concentrations were highest in E1 ($p < 0.0110$), and the $NH_4^+$ concentrations in the dry season did not differ significantly between E2 and CE, with higher mean values than in E1 ($p = 0.0008$).

In the rainy season, the $NH_4^+$ concentrations were highest in E2 soils ($p < 0.0001$). When the mineral N means were compared between the dry and rainy seasons, there was a

significant difference for all areas, which was highest in the dry season, E1 ($p = 0.0056$), E2 ($p = 0.0025$) and CE $P = 0.0036$) for $NO_3^-$ and for $NH_4^+$, E1 ($p = 0.0024$), E2 ($p = 0.0002$) and CE ($p = 0.0009$) in 2 cm deep soil.

### 3.3. RELATIONSHIPS BETWEEN SOIL $CH_4$ AND $N_2O$ FLUXES AND ENVIRONMENTAL VARIABLES

The axes of the principal components (PC1 and PC2) help to visualize trends of the environmental variables (WFPS, $NO_3^-$, $NH_4^+$, and soil temperature) and of $CH_4$ and $N_2O$ fluxes per year (Figure 2.4). According to the Principal Component Analyses (PCA) of the environmental variables, in year-1, the sum of the axes for $CH_4$ was 58 % (PC1 34 % and PC2 24 %) and in year-2 was of 54 % (PC1 32 % and PC2 22 %) (Figures 2.4a and b).

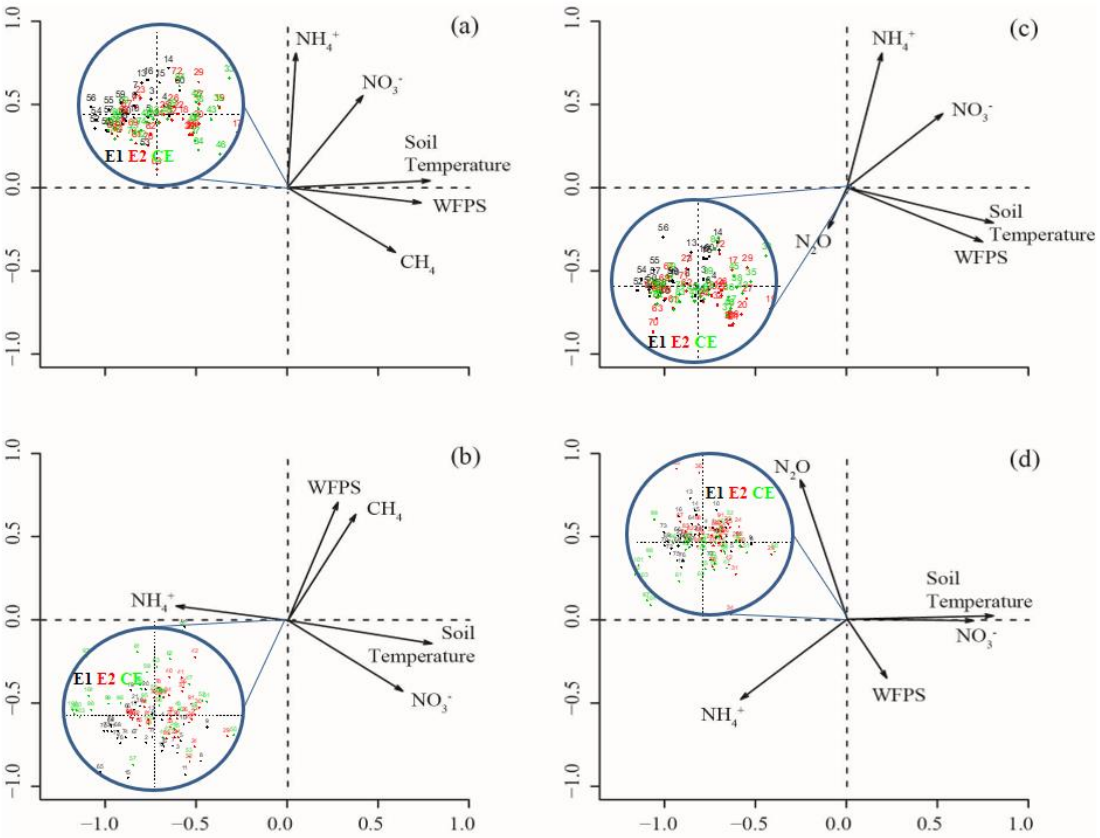


**Figure 2.4** - Analysis of principal components related to environmental variables and $CH_4$ (a) year-1 and (b) year-2; and $N_2O$ (c) year-1 and (d) year-2 in *Eucalyptus* stands E1 (planted in 2011, clone EAC 1528) and E2 (planted in 2009, clone GG100) and CE (native cerrado vegetation), Distrito Federal, Brazil.

For $N_2O$, in year-1, the two first axes were 51 % (PC1 30 % and PC2 21 %), and in year-2, 52 % (PC1 31 % and PC2 21 %) (Figures 2.4c and d). The environmental variables in year-1 showed a higher clustering tendency than in year-2 (Figure 2.4). There was a trend of clustering $CH_4$ fluxes mainly with WFPS, independent of the year (Figures 2.4a and b) and in year-1 also with the soil temperature (Figure 2.4a). No defined clustering trend for $N_2O$ fluxes with the environmental variables was observed in either year (Figures 2.4c and d).

The weights of the environmental variables in the linear combination that define each PCA are shown in Table 2.2. In year-1, PC1 presented correlations as a function of the axis of 0.59 for $CH_4$ flux and greater than 0.74 for soil temperature and WFPS, but the highest correlation as a function of the axis occurred in PC2 for $NH_4^+$, where groups of positive correlations were observed (Figures 2.4a, b and Table 2.2).

The graphical distribution and weights of evidence established by the PCA for $N_2O$ were similar to the PCA for $CH_4$ in the same year, but the correlation of $N_2O$ in function of the axis was low in PC1 and PC2 (Table 2.2). In the year-2, PC2 showed a higher correlation in function of the axis with $CH_4$ (0.64) and WFPS (> 0.70), while soil temperature had the highest observed correlation of 0.79 in PC1 (Figure 2.4c and Table 2.2). Different from year-1, PC2 indicated a high correlation for $N_2O$ as a function of the axis and did not cluster with environmental variables, although good correlations (0.70 and 0.80) were observed for $NO_3^-$ and soil temperature, respectively, on PC1 (Figure 2.4 and Table 2.2).

**Table 2.2 -** Correlation between each principal component and environmental variables as a function of years 1 and 2.

| Variables | Year-1 | | | | Year-2 | | | |
|---|---|---|---|---|---|---|---|---|
| | **PC1** | **PC2** | **PC1** | **PC2** | **PC1** | **PC2** | **PC1** | **PC2** |
| | **$CH_4$** | | **$N_2O$** | | **$CH_4$** | | **$N_2O$** | |
| GHG | 0.59 | -0.39 | -0.10 | -0.25 | 0.37 | 0.64 | -0.26 | 0.84 |
| WFPS | 0.74 | -0.09 | 0.74 | -0.33 | 0.28 | 0.71 | 0.22 | -0.36 |
| Soil Temp. | 0.79 | 0.04 | 0.80 | -0.21 | 0.79 | -0.14 | 0.80 | 0.02 |
| $NO_3^-$ | 0.42 | 0.55 | 0.52 | 0.44 | 0.63 | -0.43 | 0.70 | -0.01 |
| $NH_4^+$ | 0.05 | 0.81 | 0.19 | 0.81 | -0.61 | 0.09 | -0.59 | -0.48 |

## 3.4. CUMULATIVE FLUXES OF $CH_4$, $N_2O$, CARBON EQUIVALENT (C eq) AND GLOBAL WARMING POTENTIAL

The cumulative fluxes were not influenced by the annual variation, age of the *Eucalyptus* stands, nor by the replacement of native vegetation by *Eucalyptus*, considering the time interval since the beginning of evaluation (Figure 2.5). In the two years of evaluation, there were no significant differences among areas and between years with regard to cumulative $CH_4$ and $N_2O$ fluxes. For $N_2O$, the variations were 0.33 ± 0.20 at 0.85 ± 0.45 kg $ha^{-1}$ $yr^{-1}$ in year-1 and 0.32 ± 0.08 at 0.43 ± 0.09 kg $ha^{-1}$ $yr^{-1}$ in year-2 (Figures 2.5a and c).

With regard to $CH_4$, the values were negative, with accumulated influxes of -1.86 ± 1.36 to -0.63 ± 0.53 kg $ha^{-1}$ $yr^{-1}$ in year-1 and -1.85 ± 1.4 to -1.34 ± 0.36 kg $ha^{-1}yr^{-1}$ in year-2 (Figures 2.5b and d). Assuming a global warming potential of $N_2O$, the C eq ranged from 42 to 108 kg $ha^{-1}$ in year-1 and from 40 to 54 kg $ha^{-1}$ in year-2 (Figures 2.5a and c). While the accumulated annual C eq referring to $CH_4$ was negative in all areas evaluated, ranging from -6 to -17 kg $ha^{-1}$ in year-1 and -12 to -17 kg $ha^{-1}$ in year-2 (Figures 2.5b and d).

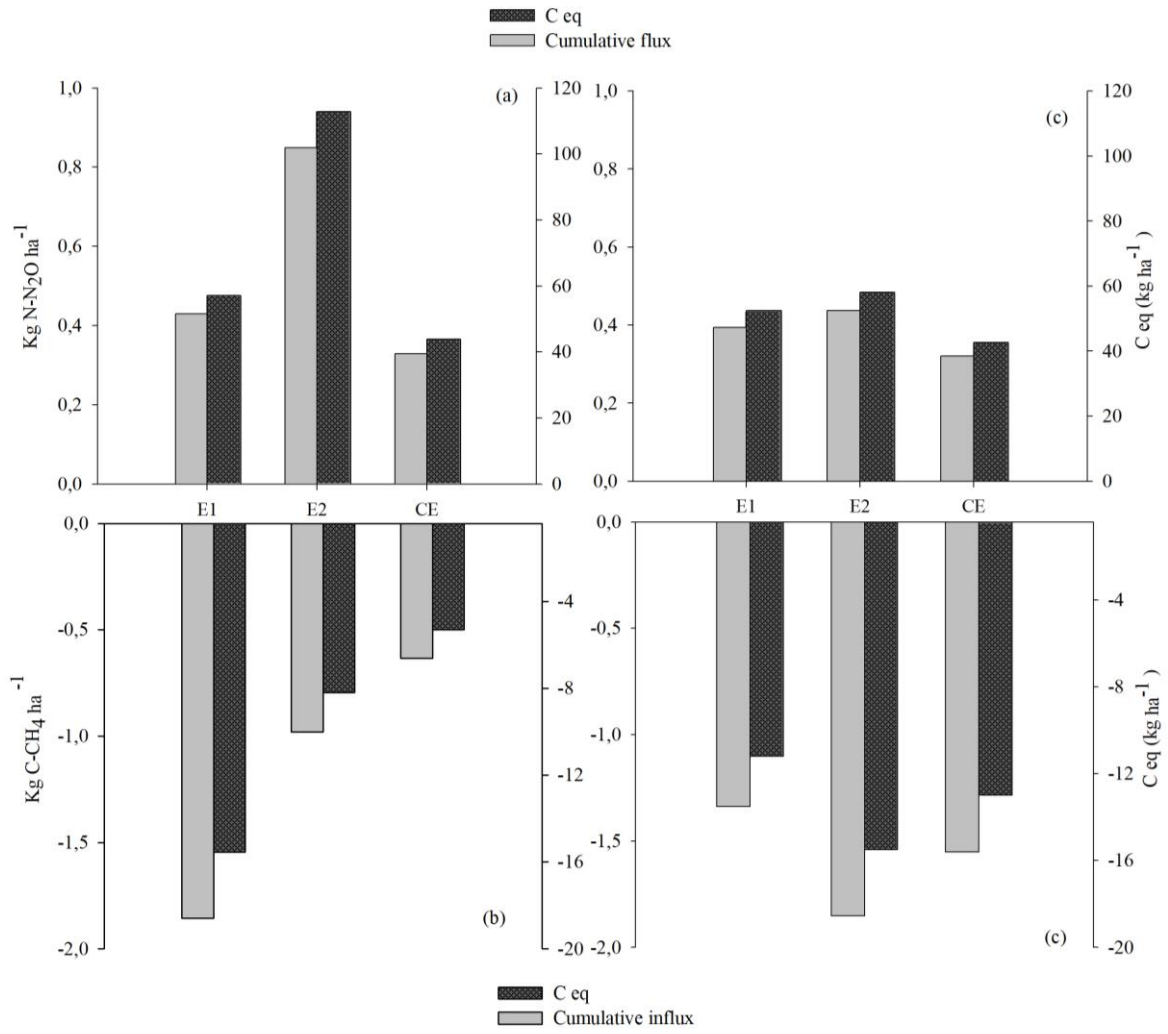


**Figure 2.5 -** Cumulative fluxes and Carbon equivalent (C eq) in year-1 (October 2013 to September 2014) of $N_2O$ (a) and $CH_4$ (b); and in year-2 (October 2014 to September 2015) of $N_2O$ (c) and $CH_4$ (d) in *Eucalyptus* stands E1 (planted in 2011, clone EAC 1528) and E2 (planted in 2009, clone GG100) and CE (native cerrado vegetation), Distrito Federal, Brazil.

The contributions of the $CH_4$ and $N_2O$ fluxes of the global warming potential (GWP) were 82 ± 80, 228 ± 140 and 82 ± 48 kg $CO_2$ eq $ha^{-1}$ $year^{-1}$ (year-1) and 84 ± 61, 84 ± 10 and 57 ± 44 kg $CO_2$ eq $ha^{-1}$ $year^{-1}$ (year-2) for E1, E2 and CE, respectively. Considering the annual GWP, $CH_4$ had a negative contribution to all studied areas (Figure 2.6) with -46 ± 34, -24 ± 23 and -16 ± 13 kg $CO_2$ eq $ha^{-1}$ $year^{-1}$ (year-1) and -33 ± 9, -46 ± 35 and -38 ± 42 kg $CO_2$ eq $ha^{-1}$ $year^{-1}$ (year-2) for E1, E2 and CE respectively.

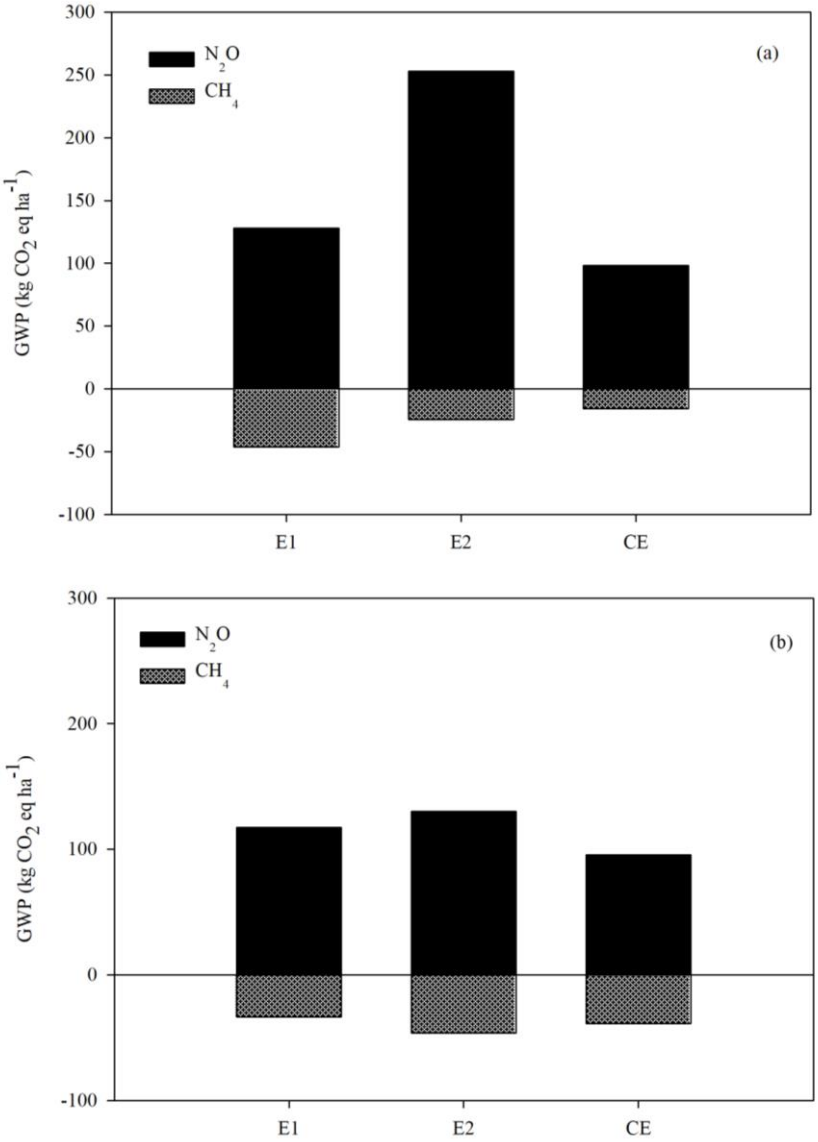


**Figure 2. 6 -** Contribution of the $CH_4$ and $N_2O$ gas to the Global Warming Potential (GWP).* year-1 (October 2013 to September 2014) and year-2 (October 2014 to September 2015).

The contribution of the $N_2O$ flow to the annual GWP was positive 128 ± 78, 253 ± 135 and 98 ± 60 kg $CO_2$ eq $ha^{-1}$ $year^{-1}$ (year-1) (a) and 117 ± 53, 130 ± 26 and 95 ± 23 kg $CO_2$ eq $ha^{-1}$ $year^{-1}$ (year-2) (b) for E1, E2 and CE respectively. The GWP had a contribution of the $N_2O$ flow above 70 % for the two years studied. Although E2 presents the upper GWP in relation to other areas, no significant differences were observed between the environments for two years of monitoring.

# 4. DISCUSSION

## 4. 1. TEMPORAL VARIABILITY OF $CH_4$ AND $N_2O$ FLUXES AND INFLUENCE OF ENVIRONMENTAL FACTORS

The fluxes negative of $CH_4$ obtained in this study (81% for E1 and E2, and 57 % for CE) are expected in forest. In general, well-drained soils consume atmospheric $CH_4$ (Ciais et al., 2013; IPCC 2013) and these negative numbers indicate $CH_4$ uptake from the atmosphere mediated essentially by methanotrophic bacteria driven by reduced soil water content, resulting in a facilitated diffusion of atmospheric $CH_4$ into soils (Hiltbrunner et al., 2012; Godoi et al. 2016). Other studies focused on savannas also mentioned this uptake condition. For *Eucalyptus globulus* stands and Australian savanna vegetation, respectively, Livesley et al. (2009) recorded -7 and -16 μg $m^{-2}$ $h^{-1}$. In areas under cerradão, Siqueira-Neto et al. (2011) reported $CH_4$ fluxes negative between -93 and -29 μg $m^{-2}$ $h^{-1}$.

The peak of $CH_4$ fluxes for the three areas occurred in the dry season. Although the WFPS did not indicate high water saturation rates. Priano et al. (2014) found an inverse relation entre $CH_4$ emission rate and WFPS. Sey et al. (2008) suggested that an anaerobic environment in the whole soil is not a condition for $CH_4$ soil emissions, since methanogenesis processes can occur at specific microsites with $O_2$ deficits, especially within soil aggregates.

The results of this study also indicate that wet soils have the capability to oxidize as much $CH_4$ as relatively dry soils, for having between 10 and 70 % WFPS. Previous reports also demonstrated that peak rates of $CH_4$ soil oxidation occur at some intermediate level of soil water content (Bender and Conrad, 1995; Del Grosso et al., 2000). According to Humer and Lechner (1999), the optimum saturation degree for oxidation is between 40 and 80 % (moisture content between 25 and 50 %). Moreover, measuring potential methane ($CH_4$) oxidation rates in semiarid soil, Sullivan et al. (2013) observed higher $CH_4$ oxidation rates in the wet than the dry season.

In the same time interval, $N_2O$, fluxes greater than 10 μg $m^{-2}$ $h^{-1}$ were rarely observed. These results were similar to previous descriptions in studies on native cerrado vegetation, in Oxisols, which reported few measurements above 10 μg $m^{-2}$ $h^{-1}$ and mean fluxes between 0.6 and 16 μg N-$N_2O$ $m^{-2}$ $h^{-1}$ (Siqueira-Neto et al., 2011; Cruvinel et al., 2011; Carvalho et al., 2017; Santos et al., 2016).

Forest soils in the Cerrado biome usually have low $N_2O$ fluxes, which can be attributed to the physical, chemical and biological characteristics of the system (Cruvinel et

al. 2011; Martins et al., 2015b; Santos et al., 2016). In general, N availability in tropical savanna ecosystems in Brazil is low, since 15 to 37 % of N is resorbed prior to leaf dehiscence (Nardoto et al., 2006) and in the Cerrado, inorganic N is made available by mineralization of soil organic matter (Catão et al., 2016). Based on the assumption that under undisturbed conditions, pools are at steady state and production and consumption are equal (Booth, 2005), the annually mineralized inorganic N in unburned Cerrado does not exceed 15 kg $ha^{-1}yr^{-1}$ (Nardoto et al., 2006). With these efficient cycling and use mechanisms, little is lost through leaching or gas transformation (Bustamante et al., 2006).

In Cerrado soils under natural conditions, the available N depends to a large extent on organic sources, such as organic matter and litter. The equilibrium between mineralization and immobilization depends on the C:N ratio, which in this study is high (> 65: 1) (Table 2.1) and on the material being incorporated into the soil, which may induce N immobilization, showing that the residue quality also influences $N_2O$ emissions. According to Alluvione et al. (2010), a high C:N ratio may increase N immobilization, thus reducing the occurrence of denitrification and consequently of GHG emissions.

In this sense, Lopes and Cox (1977) attributed the observed $N_2O$ influxes into native vegetation to low soil pH values. In fact, Catão et al. (2016) found a significantly lower abundance of archaeal (AOA) and bacterial (AOB) ammonia oxidizers in a low-pH, undisturbed Cerrado soil than in an adjacent site converted to agriculture management. Cerrado soils can be flooded during short moments in the rainy season, but they are often described as well-drained, leached and oligotrophic soils (Ribeiro and Walter, 2008).

Although denitrification can occur in aerated soils (Braker et al., 2015), it is not expected to be intense in the Cerrado, especially because of the low accumulation of $NO_3^-$ in these soils, which could be interpreted as $NH_4^+$ assimilation by the vegetation. This is confirmed by the high percentage of ammonia assimilation genes (37 %) annotated in the metagenomes as well as by the low abundance of ammonia oxidizers in undisturbed Cerrado soils (Catão et al., 2016). Low abundance of archaeal and bacterial ammonia oxidizers in Cerrado soils may also occur due to competition with soil fungi for ammonium (Yu et al., 2014). Surveys in Cerrado soils indicate that arbuscular mycorrhizal fungi occur in a large number of native plants and phytophysiognomies (Assis et al., 2014) and interact with commercial crops, e.g., *Eucalyptus* plantations (Campos et al., 2011).

In general, in the combination of good drainage conditions, which reduces WFPS (Baggs and Philippot, 2010), with low relative $NO_3^-$ production, the mineral N concentrations rarely exceed the N demand for microorganisms and plant roots (Nardoto and

Bustamante, 2003; Martins et al., 2015b). Although several studies reported correlations between GHG and mineral N in the soil (Siqueira-Neto et al., 2011; Santos et al. 2016; Carvalho et al., 2017), and in this study we also observed some correlations between these N forms and gas fluxes, it is worth emphasizing that the gas emission pulses did not occur synchronously with the highest $NO_3^-$ and $NH_4^+$ concentrations, which may have reduced the significance of the relations.

However, it should be considered that the transformations of N in an ecosystem (immobilization or mineralization) are coupled to C transformations, especially when organic carbon molecules are converted into $CO_2$ by soil heterotrophic microbial populations (McGill and Cole, 1981), which can reduce the partial pressure of oxygen and favor denitrification. Significant $N_2O$ pulse emissions following the first rains after a dry season, often with a small time lag, have been reported for different seasonally dry ecosystems and described by Werner et al. (2014), being generally preceded by significant $CO_2$ emissions immediately after the soil is re-moistened, due to water-induced activation of soil microbes.

In this study, low GHG fluxes and influxes were observed, regardless of the season and years studied, which were predominantly associated with WFPS < 50 % and magnitudes of $NO_3^-$ concentrations below 8 mg $kg^{-1}$. In soils with high permeability, as in the case of the Latosol of this study, both WFPS and texture are key factors in $N_2O$ emission. In soils with WFPS> 60 %, denitrification tends to be the predominant process (Gregorich et al., 2015; Livesley et al., 2009), while in low WFPS soils, ammonia oxidation is favored (Bateman and Baggs, 2005).

Even though denitrification usually takes place when heterotrophic bacteria under oxygen limitation use $NO_3^-$ as an alternative electron acceptor to produce $N_2O$ and $N_2$, in soils under aerobic conditions, as a function of the aerobic-anaerobic interface within the soil aggregates, the production of $NO_3^-$ may occur associated with aerobic denitrification after the anaerobic pre-phase, also known as nitrifier denitrification (Kool et al., 2011; Morley and Baggs, 2010).

It has been shown elsewhere that soil moisture expressed by WFPS, soil temperature and mineral N content are the main variables that control and express GHG emissions (Ball et al., 2014; Bayer et al., 2015; Petitjean et al., 2015; Yang et al., 2015). In this study, no significant differences were observed within each areas with regard to GHG fluxes under seasonal influence. Different observation were reported for native cerrado vegetation by Siqueira-Neto et al. (2011), where lower $N_2O$ emissions were observed during the dry

season, indicating that the effects of seasonal variation occur due to the absence of rainfall and consequent reduction in soil moisture.

This study is not showing consistent environmental 'driver' variables for the GHG fluxes. In contrast, the exactly same methods of measurements and detections were used by our research group that found significate relations among GHG and environmental/management variables in controlled agropastoral experiments (Carvalho et al., 2017; Santos et al., 2016; Sato et al., 2017). Therefore, these results indicate that the lack of consistent environmental effects may be due to both the intrinsic low GHS emission and the high spatial / temporal variability of native and cultivated forests environments in the cerrado.

## 4.2. CUMULATIVE $CH_4$ AND $N_2O$ FLUXES

The cumulative annual $CH_4$ fluxes were negative for the areas and years studied (Figures 2.5b and d). These cumulative influxes of $CH_4$ in *Eucalyptus* plantations and native savanna vegetation are in agreement with reports of emissions from forests and savannas (Table 2.3) that indicate $CH_4$ uptake from the atmosphere, since a greater amount was consumed by the environment than the effectively produced quantity (Bustamante et al., 2012; Zanoni et al., 2015).

The $CH_4$ absorption is controlled by the diffusion rate influenced by physical factors and the biological demand associated with the physical-chemical environment. Studies have indicated that the soil N mineral content may stimulate $CH_4$ oxidation (Bodelier and Laanbroek, 2004; LeBauer and Treseder, 2008; Liu et al., 2009). Mineral N seems to be a prerequisite for the occurrence of $CH_4$ consumption, although, the identification of the relationships between N availability and $CH_4$ consumption, as well as the bacteria involved, still represent a challenge. While research is underway, N must be treated as a potential inhibitor and as a beneficial factor for $CH_4$ consumption in soils (Bodelier and Laanbroek, 2004).

The emission estimates were below 0.86 kg $ha^{-1}yr^{-1}$ for all areas studied (Figures 2.5a and c). Other studies also reported cumulative $N_2O$ fluxes below 1 kg $ha^{-1}yr^{-1}$ (Table 2.3). There are no studies in Brazil that have evaluated soil $N_2O$ fluxes for a period of two years under forest ecosystems planted with eucalyptus. In our study, $N_2O$ influx was mainly observed in the native Cerrado during the dry season (WFPS<47%) and low nitrate levels (Figures 2.3a e b). The influx of $N_2O$ under native vegetation may be associated with a low N mineral content, in predominantly ammoniacal-nitrogen form, a rapid drainage of soil

water to the subsurface layers (Martins et al., 2015b; Santos et al., 2016; Carvalho et al., 2017; Sato et al., 2017), low soil pH (Lopes and Cox,1977), since nitrification tends to decrease with increasing soil acidity (Hickman et al., 2014).

Lower $N_2O$ fluxes were observed by Carvalho et al. (2017) in a crop-livestock integration system forest, when compared to the integration system of livestock farming and this is due, according to the authors, to the presence of eucalyptus in the production system, which plant residues are rich in phenolic compounds and low pH (Soumare et al., 2015), inhibiting microbial and enzymatic activity in soil (Chen et al., 2013). In addition, the rapid growth of eucalyptus provides better microclimate and high C:N ratio (Table 2.1), contributing to the low accumulated GHG fluxes of this species.

**Table 2.3 -** Literature review of annual or partial rates of C-$CH_4$ and N-$N_2O$ fluxes from soils under forests.

| Country | Areas | C-$CH_4$ kg $ha^{-1}$ | N-$N_2O$ kg $ha^{-1}$ | Reference |
|---|---|---|---|---|
| Brazil | Woodland | - | -0.50 | Carvalho et al. (2017) |
| Brazil | Woodland | - | 0.07 | Silva et al. (2017) |
| Brazil | Cerrado *stricto sensu* | - | 0.55 | Sato et al. (2017) |
| Brazil | Cerrado *stricto sensu* | - | 0.28 | Santos et al. (2016) |
| Brazil | Cerrado *stricto sensu* | -4.4 | 0.40 | Carvalho et al. (2014) |
| Brazil | *Eucalyptus urograndis* | - | 0.70 | Coutinho et al. (2010) |
| Africa | Savanna vegetation | - | 2.33 | Castaldi et al. (2013) |
| Australia | Native Forest (Savanna) | -1.6 | 0.02 | Grover et al. (2012) |
| Australia | Native Forest (Savanna) | -1.4 | 0.16 | Livesley et al. (2009) |
| Australia | *Eucalyptus globulus* | -6.8 | 0.15 | Livesley et al. (2009) |
| Australia | *Pinus radiata* | -5.0 | 0.12 | Livesley et al. (2009) |

The intensity of nitrification and denitrification processes varies according to the $NH_4^+$ and $NO_3^-$ soil concentrations, as suggested by Dobbie and Smith (2003). However, Chantigny et al. (1998) mentioned that in hot and humid regions, both processes only respond positively to the substrate when the mineral N content exceeds 5 mg N $kg^{-1}$. In this study, 76 % and 42 % of the mineral N concentrations were higher than 5 mg N $kg^{-1}$ in year-1 and year-2, respectively. Based on these findings, it can be concluded that eucalypt stands of different ages and areas of native cerrado vegetation, in stages without any more external inputs of nitrogen sources, but depending exclusively on the cycling of the system, probably contribute to reduce $N_2O$ emissions.

Planting *Eucalyptus* in areas of former native vegetation or previous agricultural use resulted in no marked differences in relation to the cumulative GHG fluxes. From the ecological and environmental point of view, it is necessary to emphasize the greater biodiversity in areas of native vegetation (Table 3). This similarity in the cumulative GHG fluxes may be related to the stability observed in areas with eucalypt stands after planting. However, the fluxes were influenced by seasonality and a limited N supply, as well as by interactive effects with environmental variables, which can define the flux behavior and magnitude over time (Aini et al., 2015).

The GWP that refers to the potential of $CH_4$ and $N_2O$ that is used to assess the impact of the implemented systems in the greenhouse effect. From the introduction of eucalyptus plantations in the areas, its establishment did not show significant differences in relation to GHG flows, expressed by the GWP (Figure 2.6). This may be related to the similarity between the edaphoclimatic conditions of the studied environments.

## 5. CONCLUSIONS

The change in land use from Cerrado to *Eucalyptus* plantations did not induce significant changes in relation to GHGs, compared to the native vegetation. The temporal variations in GHGs fluxes and the different ages of the stands did not imply in significant differences in the cumulative annual fluxes.

The analysis of the two-year records indicated $CH_4$ uptake from the atmosphere in eucalypt stands and native savanna areas. This assessment suggests that the highest $CH_4$ and $N_2O$ pulses occurred on only a few days a year, and that they are transient and therefore have little impact on total emissions. Our results can be an important contribution to a better understanding of the dynamics of GHGs fluxes in commercial forest plantations in the Cerrado region.

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