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SAO PAULO STATE UNIVERSITY - UNESP
SCHOOL OF AGRICULTURAL AND VETERINARIAN SCIENCES
CAMPUS OF JABOTICABAL

**GREENHOUSE GAS IN AGROECOSYSTEMS:
INTEGRATION OF BIOGEOCHEMICAL PROCESSES,
PRODUCTION SYSTEMS, AND METHODOLOGICAL
CHALLENGES**

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SAO PAULO STATE UNIVERSITY - UNESP
SCHOOL OF AGRICULTURAL AND VETERINARIAN SCIENCES
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Jaboticabal, 09 de abril de 2026.

AUTHOR'S CURRICULUM VITAE

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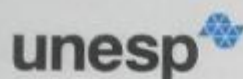
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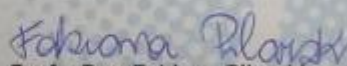
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CERTIFICADO

Certificamos que o projeto de pesquisa intitulado "**Impacto da biodiversidade de espécies em sistemas integrados sobre aspectos ambientais e produtivos de pastagens no Cerrado e Mata Atlântica**", protocolo nº 2452/21, sob a responsabilidade da Profa. Dra. Ana Cláudia Ruggieri, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 28 de outubro de 2021.

Vigência do Projeto	01/11/2021 a 30/11/2023
Espécie / Linhagem	Bovino/Nelore
Nº de animais	12 animais
Peso / Idade	210 kg / 15 meses
Sexo	Machos inteiros
Origem	Produtor-parceiro

Jaboticabal, 28 de outubro de 2021.


Profa. Dra. Fabiana Pilarski
Coordenadora – CEUA

GREENHOUSE GAS EMISSIONS IN AGROECOSYSTEMS: INTEGRATION OF BIOGEOCHEMICAL PROCESSES, PRODUCTION SYSTEMS, AND METHODOLOGICAL CHALLENGES

ABSTRACT: Mitigating greenhouse gas emissions from tropical agroecosystems requires consistent quantification and a mechanistic understanding of the processes controlling soil fluxes, particularly nitrous oxide (N_2O), whose emissions are highly variable in time and space. This research integrates three complementary lines of evidence to reduce uncertainty and strengthen inference for mitigation, inventories, and modelling: (i) a field study quantifying cumulative N_2O and methane (CH_4) emissions across contrasting land uses and interpreting their controls using environmental variables, mineral N, and functional genes; (ii) a quantitative synthesis based on a systematic review and meta-analysis to evaluate emission intensity in integrated systems; and (iii) a methodological assessment of N_2O time-series reconstruction, focusing on how interpolation choices affect cumulative estimates and emission factors under irregular sampling. In the field study, an integrated crop–livestock system (soybean, second-crop maize, and grass), a long-term monoculture pasture, and a native vegetation area as reference were compared over two years of monitoring. Cumulative N_2O emissions were characterized by high variability and a disproportionate contribution of high-magnitude events, reinforcing the dominant role of emission peaks in annual accounting. Overall, cumulative N_2O did not show statistically significant differences among land uses when evaluated across the experimental phases, indicating that differences in cumulative N_2O , if present, are strongly contingent on the joint occurrence of available nitrogen and environmentally favorable emission windows. For CH_4 , cumulative balances reflected alternation between emission and uptake over time, with the net signal depending on land use and environmental context, highlighting that soil CH_4 uptake can be an important component of the balance in well-drained soils. Mechanistic interpretation was strengthened by integrated analyses (PCA and Pearson correlations), which organized variability into two complementary dimensions. PCA indicated that the first component (PC_1) represents a land-use associated signature, in which native vegetation and managed systems diverge through the co-variation of mineral N forms and microbial functional potential, inferred from genes related to nitrification, denitrification, and biological N fixation. Under this framework, genes act as indicators of the soil functional state and the “potential” for microbial pathways, rather than immediate, deterministic predictors of fluxes. The second component (PC_2) summarizes a short-term environmental activation signal, associated with the hydroclimatic microenvironment that controls O_2 diffusion, the formation of anaerobic microsites, and the immediate availability of substrates, thereby modulating when the potential captured by PC_1 is expressed as N_2O emissions and alters the net CH_4 balance. Together, these results support an interpretation in which land use reorganizes functional potential and N

status, whereas the environment defines activation windows that regulate the expression of this potential into fluxes. In the meta-analysis, conducted through a systematic review and robust quantitative synthesis, N₂O emission intensity in integrated systems was evaluated in comparison with non-integrated systems. The analysis revealed consistent clustering patterns, indicating that integrated systems tend to exhibit lower emission intensity than monoculture systems and, in some contexts, also relative to native references, reinforcing the potential of sustainable intensification when assessed through efficiency-based metrics (emissions per unit of product). These findings also highlight heterogeneity among integrated arrangements and the need for context-specific stratification, avoiding simplistic extrapolations. Finally, the methodological assessment showed that time-series reconstruction choices directly influence cumulative N₂O emissions and emission factors when sampling is sparse and irregular, as in animal-excreta experiments. A data-driven framework was developed to compare interpolation methods using structural time-series metrics, a multicriteria score, and Pareto analysis. Among the evaluated methods, linear interpolation showed the best overall performance and the most consistent recovery of temporal patterns, and was selected as the most suitable approach for reconstructing N₂O time series in the analysed dataset. Overall, this research shows that integrating robust quantification, mechanistic interpretation (environment, mineral N, and microbial indicators), scale synthesis (meta-analysis), and rigorous time-series reconstruction is essential to improve N₂O and CH₄ estimates and to support mitigation decisions and the development of more representative emission factors in tropical agricultural systems.

Keywords: *emission factors; excretas; integrated crop-livestock systems; functional genes; meta-analysis; methane; nitrous oxide; time series.*

CHAPTER 1 - General considerations

1. Introduction

Mitigating climate change in the agricultural sector is one of the major contemporary scientific and policy challenges, especially in countries where emission profiles are strongly linked to land use dynamics and primary production. The Paris Agreement set the goal of limiting the increase in global mean temperature to well below 2 °C, while pursuing efforts to restrict it to 1.5 °C above pre-industrial levels (UNFCCC, 2015). Achieving this target depends on sustained reductions in greenhouse gas (GHG) emissions across all sectors of the economy, with particular attention to food production systems, which account for a substantial share of global methane (CH₄) and nitrous oxide (N₂O) emissions (IPCC, 2022).

In Brazil, the national emissions profile differs from that observed in predominantly industrialized economies, being characterized by the high contribution of the Land Use, Land Use Change and Forestry (LULUCF) and Agriculture sectors (MCTI, 2020; SEEG, 2024). Historically, the conversion of natural ecosystems to productive activities has generated large carbon dioxide (CO₂) fluxes associated with losses of carbon stocks from biomass and soils. However, as policies to curb deforestation are strengthened, the relative share of agricultural emissions tends to increase within the national inventory, progressively shifting mitigation efforts toward non-CO₂ gases.

Among these gases, CH₄ and N₂O exhibit distinct atmospheric and biogeochemical characteristics, with direct implications for their climatic relevance and for mitigation strategies. CH₄, predominantly derived from enteric fermentation in livestock systems, has a relatively short atmospheric lifetime but a high radiative impact over shorter time horizons. N₂O, in contrast, has an atmospheric lifetime exceeding a century and a high global warming potential over 20 and 100 year horizons, and is currently the main substance responsible for stratospheric ozone depletion (IPCC, 2022; Ravishankara et al. 2009). Its emissions are intrinsically associated with the intensification of the nitrogen cycle in agricultural soils, mediated by microbial nitrification and denitrification processes (Butterbach-Bahl et al., 2013).

Productive intensification has been presented as a central strategy to reconcile production growth with emissions mitigation, primarily by increasing productivity per

unit area and reducing pressure for further land use conversion (Cardoso et al., 2020; IPCC, 2022). However, intensification simultaneously alters multiple biogeochemical fluxes. Improvements in diet quality and animal performance may reduce methane emissions per unit of product, whereas increased nitrogen inputs to soils via mineral fertilization or greater excreta deposition on pastures may intensify nitrous oxide emissions (Hristov et al., 2013). Therefore, the assessment of the climate performance of intensified systems depends on the metric adopted, the time horizon considered, and the robustness of flux estimates.

The literature shows that N₂O emissions display high spatial and temporal variability and are strongly modulated by environmental conditions, nitrogen substrate availability, soil microbial structure, and management practices (Butterbach-Bahl et al., 2013). This variability is one of the main sources of uncertainty in national inventories, particularly for categories associated with excreta deposition on pastures and the use of nitrogen fertilizers (IPCC, 2019). Defining representative emission factors and adequately reconstructing time series of fluxes are therefore critical to the credibility of national estimates and to evaluating the real mitigation potential of production systems.

In this context, understanding the environmental and microbiological factors that modulate N₂O emissions in agricultural systems, as well as critically assessing methods for flux quantification and interpolation, is a fundamental step toward integrating experimental evidence, national inventories, and international climate commitments. Our study aimed to investigate the dynamics of N₂O emissions in integrated systems, analyze emission intensity across different production arrangements, and examine the influence of methodological approaches on the estimation of emission factors.

5. Conclusions

Emission factors for N_2O from dung and urine deposited on pasture sit at the interface between field measurements and greenhouse gas accounting. When fluxes are measured with manual static chambers, the evidence base is typically sparse and irregular. Under those conditions, the reconstruction used to integrate flux over time becomes a primary determinant of cumulative emissions and $\text{EF}_{3\text{PRP}}$, particularly when short-lived peaks carry a large share of the seasonal signal.

In this study we presented a workflow to treat gap filling as a fit-for-purpose decision for EF estimation rather than a purely statistical optimisation. The framework combines structural descriptors of reconstructed time series with complementary multi-

criteria tools (composite scoring and Pareto-front selection), and it pairs method comparisons with percentile-based event thresholds to make peak dependence explicit. We benchmarked deterministic interpolation, statistical imputation, and a dynamic Kalman filter approach on irregular N₂O series from sheep and goat dung and urine patches.

Across treatments, conservative deterministic reconstructions produced stable EF estimates under low to intermediate event thresholds, while more flexible imputations increasingly concentrated emissions into reconstructed peaks as thresholds tightened. In this sparse-sampling regime, added model flexibility did not consistently translate to more reliable EF outcomes, because event timing and persistence were often weakly constrained by observations.

The Kalman filter frequently provided a balanced middle ground, retaining more temporal structure than simple interpolation without introducing the same degree of unsupported high-frequency variability characterised in some imputations, but no single method was dominant across all conditions. Across excreta sources, urine treatments, particularly goat urine, produced higher and more variable EFs than dung treatments, indicating that reconstruction sensitivity is most critical where N inputs are concentrated and short-lived peaks dominate.

The practical implication is that EF reporting should document, rather than conceal, the dependence on reconstruction choices. For inventory-oriented applications we suggest reporting the gap structure, bracketing EF estimates with at least one conservative method alongside a time-aware alternative, and reporting sensitivity across percentile thresholds and associated event spacing (MaxGap) to show how much inference rests on a limited number of peaks. Extending this workflow across sites, years and sampling designs, and testing covariate-informed and hybrid strategies, will strengthen guidance on when complexity adds information and when it mainly adds assumptions. These steps support more defensible EF_{3PRP} estimation and more efficient monitoring designs for grazed livestock systems.

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