Journal Full Title: Journal of Biomedical Research & Environmental Sciences
Journal Website Link: https://www.jelsciences.com
Journal ISSN: 2766-2276
Category: Multidisciplinary
Subject Areas: Medicine Group, Biology Group, General, Environmental Sciences
Topics Summation: 133
Issue Regularity: Monthly
Review Process: Double Blind
Time to Publication: 21 Days
Indexing catalog: IndexCopernicus ICV 2022: 83.03 | GoogleScholar | View more
Publication fee catalog: Visit here

DOI: 10.37871 (CrossRef)
Plagiarism detection software: iThenticate
Managing entity: USA
Language: English
Research work collecting capability: Worldwide
Organized by: SciRes Literature LLC
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Forage Grass in Static Gas Exchange Chambers Deployed In Southern Amazon Influences Field Measurement of Soil $\text{N}_2\text{O}$ Emissions

Alexandre Ferreira do Nascimento$^{1,2,*}$ and Anderson Ferreira$^2$

$^1$Embrapa Agrossilvipastoril, Rodovia dos Pioneiros MT 222, Km 2.5, Zona Rural, 78550-970, Sinop, MT, Brazil
$^2$Embrapa Trigo, Rodovia BR 285, Km 294, Zona Rural, 99050-970, Passo Fundo, RS, Brazil

*Corresponding author(s)
Alexandre Ferreira do Nascimento,
Embrapa Agrossilvipastoril, Rodovia dos Pioneiros MT 222, Km 2.5, Zona Rural, 78550-970, Sinop, MT, Brazil
Email: alexandre.nascimento@embrapa.br
DOI: 10.37871/jbres1867
Submitted: 10 December 2023
Accepted: 09 January 2024
Published: 10 January 2024
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Abstract

Static chambers are used to estimate the exchange of greenhouse gases between the soil and the atmosphere, but the presence of plants inside such chambers can alter gas fluxes. This study aimed to determine the influence of forage grass on $\text{N}_2\text{O}$ fluxes emanating from an oxisol in the southern Amazon region of Brazil. A randomized experiment comprising two treatments, namely static gas exchange Chambers with Grass (CWG) and Chambers with No Grass (CNG) with six replicates of each was performed to determine $\text{N}_2\text{O}$ fluxes over a period of one year. Soil $\text{N}_2\text{O}$ fluxes in the CWG were higher (19.08 $\mu$g N$_2$O-N m$^{-2}$ h$^{-1}$) than those in the CNG (9.05 $\mu$g N$_2$O-N m$^{-2}$ h$^{-1}$), most especially during the wet season. Cumulative $\text{N}_2\text{O}$ emissions were 1.60 and 0.72 kg N$_2$O-N ha$^{-1}$ for the CWG and CNG, respectively. The higher $\text{N}_2\text{O}$ estimates in the CWG may be attributed to the plant transpiration stream and/or to changes in soil attributes induced by the plants. Measurement of $\text{N}_2\text{O}$ emissions from a grass-covered oxisol inside gas exchange chambers may overestimate soil $\text{N}_2\text{O}$ flux in the tropical humid climate of the Southern Amazon.

Introduction

Soil is an important source of Nitrous Oxide (N$_2$O), a potent gas impacting atmospheric radiation balance and ozone chemistry, having a warming potential 298 times greater than CO$_2$ [1]. Field measurements of N$_2$O emissions from soils are normally performed by collecting headspace air samples from chambers deployed on the soil surface in order to quantify soil-atmosphere gas exchange over time [2]. Although the methodology has improved during the last decade or so, the effects exerted by plants inside the chambers on the flux and cumulative emission of N$_2$O remain unclear [3].

According to Chang C, et al. [4], although plants do not generate N$_2$O within their tissues, the N$_2$O formed from nitrogen (N) by various processes in the soil is absorbed by plant roots and subsequently released via the transpiration system [4]. Apparently, the amount of N$_2$O released by plants may account for some 25 to 30 % of the total emission of this gas from the soil [5,6]. Considering that plant root exudates can affect N mineralization in soils [7,8], it is likely that the presence of living plants...
inside a gas exchange chamber would alter the flux of N\textsubscript{2}O from the soil. In this context, Vázquez E, et al. [9] observed high rates of ammonification in soil cultivated with \textit{Urochloa} genotypes in tropical pastures, indicating that plants have a modulating effect on N mineralization. Along with chemical changes, the soil surrounding roots can undergo physical modifications [10,11], and these may also play important roles in the regulation of N processes in the soil [1].

In light of the above, the aim of this study was to determine the influence of forage grass on the N\textsubscript{2}O flux from an oxisol in the Southern Amazon region of Brazil. Our results will contribute to a better understanding of the variability of N\textsubscript{2}O fluxes at the soil–atmosphere interface. This knowledge is important because N\textsubscript{2}O is a potent greenhouse gas that is stimulated by agricultural activities, and reduction of such emissions could have a positive impact on global warming.

**Material and Methods**

Experiments were performed at the research farm of Embrapa Agrossilvipastoril located in Sinop, MT, Brazil (11°5´S, 55°30´W). According to the Köppen classification system, the climate of the region is tropical (Aw) and characterized by well-defined wet and dry seasons with mean temperatures in the range 24 to 34°C and mean annual rainfall varying between 1700 and 2200 mm. The experimental area was of flat relief with a clayey textured soil, classified as Hapludox [12] or red-yellow latosol (oxisol) according to the Brazilian Soil Classification System [13], and containing 49 % clay, 16 % silt and 35 % sand. The chemical characteristics of the top 0 to 10 cm soil layer were: pH (pH measured in suspension solution after adding deionized water in a soil:water ratio of 1:2.5.) 5.4, 2.6 % total organic C, 0.2 % total organic N, 1.6 cmol\textsubscript{c} kg\textsuperscript{-1} total exchangeable bases and 42 % base saturation.

During the period November 2016 to October 2017, N\textsubscript{2}O fluxes were measured in 2 ha of pasture formed by forage grass [\textit{Brachiaria} (Syn. \textit{Urochloa}) \textit{brizantha} cv. Marandu] and grazed at a stocking density intended to maintain a mean canopy height of 30 cm above the soil surface. The pasture received surface N fertilization (50 kg ha\textsuperscript{-1}) twice during the experimental period, in November 2016 and March 2017. The randomized experimental design comprised two treatments, namely Chambers with Grass (CGW) and Chambers with No Grass (CNG), and incorporated six replicates of each [14].

The vented static chambers (non–flow–through; non–steady–state) comprised opaque rectangular PVC boxes (0.60 m long x 0.40 m wide x 0.095 m high) [2] and were deployed at random on grass covered areas or bare soil. Samplings of headspace gases were performed in the mornings (between 08:00 and 10:00) at 7 day intervals during the wet season (November 2016 to May 2017) when gas fluxes were high, and at 14 day intervals during the dry season (June to October 2017) when gas fluxes were low [15]. Gas samples were collected from the headspace of each chamber, with the aid of a 20cm\textsuperscript{3} syringe, at 0, 20, 40 and 60 min after deployment of the chamber [2]. The internal temperature of the chamber was monitored at the time of gas collection. Air samples were transferred from the syringes to 20 cm\textsuperscript{3} evacuated glass vials capped with gray butyl rubber septa, and subsequently analyzed on a model GC–2014 chromatograph (Shimadzu, Tokyo, Japan) equipped with an electrical conductivity detector. The amounts of N\textsubscript{2}O present were established from calibration curves constructed with N\textsubscript{2}O standards of three known concentrations analyzed using the same chromatographic equipment and parameters as the samples. The results were used to adjust the linear model by relating the variations of gas concentrations in the chamber headspace as a function of time (0, 20, 40, and 60 min). Soil N\textsubscript{2}O fluxes were calculated according to Eq. (1) proposed by Hutchinson and Livingston [16]:

$$\text{Soil N}_2\text{O flux (µg N}_2\text{O-O}_2 \text{ m}^{-2} \text{ h}^{-1}) = \frac{dC}{dt} \times \frac{V}{A} \times \frac{m}{vm} (1)$$

where $dC/dt$ is the change in gas concentration in the chamber as a function of time, $V$ is the chamber volume (L), $A$ is the area of the chamber (m\textsuperscript{2}), $m$ is the molecular weight (g) and $vm$ is the molecular volume (L) of the gas. In the treatment with plants, $V$ was adjusted by subtracting the volume occupied by the plant biomass, noting that tall grasses were folded to enable them to fit within the height of the chamber [3,17].

The N\textsubscript{2}O flux values were used to estimate cumulative N\textsubscript{2}O emissions during the study period according to the numerical integration method [18]. Values of N\textsubscript{2}O fluxes determined every 7 or 14 days throughout the evaluation year were expressed together with the Standard Error of the Mean (SEM) [19]. The Pearson correlation coefficient was used to determine the relationship between soil N\textsubscript{2}O fluxes
recorded in the CWG and CNG during the evaluation period, and the best-fit linear regression equation was established in order to understand the difference between the two treatments. Cumulative emission data and mean N$_2$O fluxes for the CWG and CNG obtained during the entire evaluation period were submitted to one-way analysis of variance (ANOVA) and the significance of the difference determined from the p value ($\alpha = 0.05$). In order to verify the difference between N$_2$O fluxes in the two treatments, the mean absolute error (MAE) and root mean squared error (RMSE) were calculated using Eqs. (2) and (3), respectively:

$$MAE = \frac{1}{n} \sum (y_1 - y_2)$$

$$RMSE = \sqrt{\frac{1}{n} \sum (y_1 - y_2)^2}$$

where $y_1$ is the daily flux in the CNG, $y_2$ is daily flux in the CWG and $n$ is the number of sampling events. Values of MAE and RMSE near to zero mean that the difference between the treatments is small.

**Results**

The N$_2$O fluxes in the CWG were higher than those in their CNG counterparts on more than 80% of the sampling dates, most especially during the wet season (Figure 1). Throughout the dry season, the fortnightly N$_2$O flux values recorded in the two treatments were similar, barring two instances in August 2017 when the values diverged, while the weekly N$_2$O flux values registered during the wet season were dissimilar, except for two occasions (November 2016 and March 2017) when they converged.

During the wet season, N$_2$O fluxes ranged from 10 to around 70 $\mu$g N$_2$O-N m$^{-2}$ h$^{-1}$ in the CWG and from 5 to around 40 $\mu$g N$_2$O-N m$^{-2}$ h$^{-1}$ in the CNG, while in the dry season both chambers presented similar low N$_2$O fluxes (< 10 $\mu$g N$_2$O-N m$^{-2}$ h$^{-1}$). The mean N$_2$O flux in the CWG (19.08 $\mu$g N$_2$O-N m$^{-2}$ h$^{-1}$) was approximately double that in the CNG (9.05 $\mu$g N$_2$O-N m$^{-2}$ h$^{-1}$), and a similar pattern was observed with regard to the cumulative N$_2$O emissions, which were registered as 1.60 kg N$_2$O-N ha$^{-1}$ in the CWG and 0.72 kg N$_2$O-N ha$^{-1}$ in their CNG counterparts. The Person correlation coefficient indicated a very strong positive correlation between the N$_2$O fluxes of the CWG and CNG. The slope of the regression line of best fit (1.88) demonstrated that the presence of plants inside the gas exchange chambers increased N$_2$O flux considerably (Figure 2). Moreover, the MAE (14.34 N$_2$O-N m$^{-2}$ h$^{-1}$) and RMSE (10.05 $\mu$g N$_2$O-N m$^{-2}$ h$^{-1}$) values relating to N$_2$O fluxes in the CWG and CNG verified that there were substantial differences between the two chambers.

**Discussion**

The principal aim of the present study was to determine the influence of forage grass on N$_2$O fluxes emanating from an oxisol in a field in the Southern Amazon. All of the statistical tools applied to compare N$_2$O fluxes in chambers with and without grass were consistent with the hypothesis that the inclusion of grass plants inside static chambers resulted in higher estimations of N$_2$O emissions from the soil.
Previous studies have shown that, in addition to soil emissions, the transpiration of plants present inside gas exchange chambers may contribute to N₂O emissions [4–6]. When the emissions attributed to plant transpiration, which reportedly amount to 30% of total soil emissions [5,6], were subtracted from the cumulative N₂O emission determined in the CWG, the result (1.12 kg N₂O-N ha⁻¹) was still 60% higher than the cumulative emission observed in the CNG (0.72 kg N₂O-N ha⁻¹). This calculation demonstrates that the plants inside the chambers may influence the formation of N₂O in the soil, and that the excess emissions are not solely due to transpiration.

One hypothesis concerning the role of plant roots on the formation of N₂O emissions relates to the manner in which they change the characteristics of the soil [10,11], thereby altering the processes of N₂O formation. For example, the rise in N₂O flux could be explained by an increase in N mineralization resulting from rhizodeposition and microbial activity [8,9], together with an increase in soil bulk density around the roots [10,11]. Increased NH₄⁺ availability, caused by N mineralization, and elevated soil bulk density in the rhizosphere, are ideal conditions for the formation of N₂O under conditions of low soil redox potential (Eh) [1]. Reductions in Eh occur mainly after rainfall when water fills the soil pore spaces, a phenomenon that has been observed in pasture covered soil during the wet season in the southern Amazon [20].

It is likely that grass roots in the soil inside the CWG had opened interconnected macropores that facilitated the upward movement of N₂O [21]. On the other hand, the bare soil inside the CNG possessed no interconnected macropores and, therefore, generated low N₂O fluxes in comparison with those of the CWG. This explanation justifies the high Pearson correlation coefficient recorded despite the differences in N₂O flux values between the two chambers, and suggests that the process of N₂O formation is analogous in the CNG and CWG, although the presence of grass in the latter may have improved emission of the gas. If N₂O had not been generated by the same processes in the soil of both chambers, it is likely that fluxes in the CNG would not have increased in step with those in the CWG and the Pearson correlation coefficient would not have been so strong. The observations above support the premise that the gas originated from analogous soil processes, regardless of the chamber, although the amounts were different.

It would be interesting to investigate the influence of the plant transpiration stream on the upward movement of N₂O formed in deeper soil horizons. Nevertheless, the amount of N₂O emitted in the CWG in the present study cannot be explained by this pathway alone because N₂O formation in deep soil horizons is lower than that in surface soil [22]. However, to confirm the hypothesis outlined above, it is imperative to investigate N₂O production in the soil profile.

Considerable research effort has been devoted to the development and application of static gas exchange chambers but the results obtained have been somewhat discrepant because some studies have included plants while others have not [2]. Moreover, a number of researchers have opted to assess fluxes in chambers containing plants that were subsequently removed after reaching a certain height [3]. Although chambers with plants may lead to the overestimation of gas emissions from the soil, it is likely that chambers without plants cannot accurately represent the situation in the field [2].

Conclusion

In the humid climate of the Southern Amazon, N₂O emissions from oxisol in static gas exchange chambers containing tropical forage grass are considerably higher than those in chambers that do not contain grass. Such overestimation of N₂O flux may be attributed to the plant transpiration stream and/or to changes in soil attributes induced by the plants. Our study has provided a number of constructive pointers for future research.

Acknowledgement

This study was partially funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de Mato Grosso (FAPEMAT).

Conflicts of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article.

Author contributions

Conceptualization, Formal analysis, Investigation,
Resources, Writing – original draft Writing – review & editing – Nascimento, A.F.; Ferreira, A.; Funding acquisition, Methodology, Project administration, Supervision – Nascimento, A.F.

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