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Standardized Static-Chamber Protocol for Soil CO_2, CH_4, and N_2O Flux Measurements in Tropical Environments

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Protocolo Padronizado de Câmara Estática para Medição dos Fluxos de CO_2, CH_4 E N_2O do Solo em Ambientes Tropicais

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ABSTRACT

Static chambers are widely used to quantify soil–atmosphere exchanges of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), but methodological inconsistencies can affect flux estimates and data comparability. This Technical Note describes and standardizes the protocol adopted by the Laboratory of Research in Environmental Biogeochemistry (LAPBio, INPE) for greenhouse gas measurements in tropical soils using non-flow-through, non-steady-state static chambers. The procedure integrates chamber design and installation, discrete gas sampling at 1, 10, 20, and 30 min after closure, chromatographic determination, multipoint calibration, chamber-specific flux calculation, and quality-control criteria. Fluxes are estimated by least-squares regression using actual elapsed sampling times and corrected according to effective headspace volume, enclosed soil area, atmospheric pressure, and chamber-headspace temperature. Regression diagnostics, chromatographic performance, field records, and gas-specific minimum detectable fluxes are jointly considered during validation. The protocol provides a standardized and traceable methodological reference for future studies of soil CO_2, CH_4, and N_2O fluxes under tropical field conditions.

Keywords: gas chromatography, soil–atmosphere exchange, biogeochemistry, quality assurance, minimum detectable flux.

RESUMO

As câmaras estáticas são amplamente utilizadas para quantificar as trocas solo–atmosfera de dióxido de carbono (CO_2), metano (CH_4) e óxido nitroso (N_2O), porém inconsistências metodológicas podem afetar as estimativas de fluxo e a comparabilidade dos dados. Esta Nota Técnica descreve e padroniza o protocolo adotado pelo Laboratório de Pesquisas em Biogeoquímica Ambiental (LAPBio, INPE) para medições de gases de efeito estufa em solos tropicais por câmaras estáticas não estacionárias e sem fluxo. O procedimento integra o desenho e a instalação das câmaras, a coleta discreta de gases aos 1, 10, 20 e 30 min após o fechamento, a determinação cromatográfica, a calibração multiponta, o cálculo de fluxo específico para cada câmara e os critérios de controle de qualidade. Os fluxos são estimados por regressão linear utilizando os tempos efetivos de coleta e corrigidos segundo volume do espaço de cabeça, área do solo, pressão atmosférica e temperatura interna da câmara. A validação considera conjuntamente parâmetros de regressão, desempenho cromatográfico, registros de campo e fluxos mínimos detectáveis específicos para cada gás.

Palavras-chave: cromatografia gasosa; troca solo–atmosfera; biogeoquímica; garantia da qualidade; fluxo mínimo detectável.

INTRODUCTION

Soil–atmosphere exchanges of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) are important components of terrestrial greenhouse gas budgets and reflect the combined influence of biological activity, soil properties, water availability, temperature, vegetation, and land management. In tropical environments, these controls may vary markedly over short spatial and temporal scales, making field measurements particularly sensitive to sampling design and methodological consistency. Static chambers remain among the most widely used approaches for quantifying soil greenhouse gas fluxes because they are relatively simple to deploy, adaptable to contrasting field conditions, and suitable for replicated measurements of multiple gases.

Despite their operational advantages, chamber-based measurements are susceptible to methodological artifacts. Chamber geometry, insertion depth, effective headspace volume, sealing, pressure equilibration, closure duration, sampling frequency, temperature variation, and gas withdrawal procedures can modify the concentration–time relationship used for flux estimation (DAVIDSON et al., 2002; COLLIER et al., 2014; CLOUGH et al., 2020). Subsequent steps, including sample storage, chromatographic determination, calibration, regression fitting, and flux calculation, introduce additional sources of uncertainty. Consequently, reproducibility depends not only on chamber design but on the integration

of field, analytical, computational, and quality-control procedures within a traceable workflow.

Particular attention is required when fluxes are low, negative, or close to the analytical detection capability. The use of a single statistical threshold, such as the coefficient of determination of the concentration–time regression, may lead to inappropriate exclusion of environmentally plausible observations. Flux validation should therefore consider regression diagnostics together with analytical performance, chamber conditions, field records, and gas-specific detection limits (PARKIN et al., 2012; VENTEREA et al., 2020). Likewise, the analytical limit of detection of a chromatographic system should be distinguished from the minimum detectable flux of the complete chamber–measurement system, which also depends on chamber geometry, sampling duration, and analytical variability.

The Laboratory of Research in Environmental Biogeochemistry (LAPBio), National Institute for Space Research (INPE), Brazil, has applied non-flow-through, non-steady-state static chambers for simultaneous determination of CO_2, CH_4, and N_2O fluxes in tropical soils. The protocol integrates chamber construction and deployment, discrete gas sampling, chromatographic analysis, calibration, chamber-specific flux calculation, and explicit quality-assurance criteria. The chamber system, field procedures, chromatographic configuration, calibration strategy, flux calculations, and validation framework are detailed throughout this Technical Note.

Accordingly, this Technical Note aims to document and standardize the LAPBio static-chamber methodology for determination of soil–atmosphere CO_2, CH_4, and N_2O fluxes, providing a reproducible reference for field measurements, chromatographic analysis, flux calculation, and quality control in tropical soils.

Static Chamber Method and Field Protocol

The method adopted by LAPBio is based on a non-flow-through, non-steady-state static chamber system, in which soil–atmosphere gas exchange is estimated from changes in gas concentration within a defined headspace during a controlled chamber-closure period. The protocol was established for the simultaneous determination of CO_2, CH_4, and N_2O fluxes under tropical field conditions and integrates chamber design and deployment, gas sampling and storage, and measurements of the environmental and physical variables required for flux calculation.

Particular attention is given to chamber geometry, sealing, pressure equilibration, effective headspace volume, closure duration, and sampling procedures because these factors can influence the concentration–time relationship from which gas fluxes are estimated (CLOUGH et al., 2020). The procedures described below constitute the standard LAPBio protocol, while allowing adjustments in

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spatial replication and chamber arrangement according to site characteristics and experimental objectives.

Chamber Design and Installation

The LAPBio static chamber system consists of a PVC collar installed in the soil before sampling and a removable polypropylene lid used to close the chamber during gas collection. The collar has a nominal internal diameter of 40.0 cm, a height of 7.5 cm, and a wall thickness of 1.0 cm. The polypropylene lid has a truncated-conical geometry, with a height of 28.0 cm, an upper radius of 16.0 cm, and a lower radius of 19.5 cm. The lid has an internal volume of approximately 27.8 L. Because the collar is inserted 2.5–5.0 cm into the soil, the exposed portion of the collar contributes additional headspace, resulting in a typical effective chamber volume of approximately 31–34 L. The effective headspace is therefore determined individually for each chamber at the time of sampling from the chamber geometry and the measured exposed collar height. The main dimensions and components of the chamber system are presented schematically in figure 1.

The contact surfaces between the collar and lid are machined to improve fitting and sealing. A polytetrafluoroethylene (PTFE) sealing strip is applied along the contact surface to minimize gas leakage. The external surface of the lid is covered with a reflective aluminum thermal blanket to reduce radiative heating and consequent changes in headspace temperature during chamber closure. Two ports are installed at the top of the lid. The sampling port consists of a circular septum made of flexible transparent rubber through which the chamber gas is withdrawn with a needle. The second port functions as a pressure-equilibration vent and consists of an approximately 10-cm-long teflon tube, of which approximately 9 cm extend into the chamber headspace. The vent allows pressure within the chamber to remain close to ambient atmospheric pressure during closure and sampling, thereby reducing pressure-induced disturbances of soil–atmosphere gas exchange (CHRISTIANSEN et al., 2011; CLOUGH et al., 2020).

Before field deployment, chamber components are visually inspected and tested for leakage, with particular attention to the lid–collar interface, sealing surfaces, septum, connections, and pressure-equilibration vent. Components showing deformation, damaged sealing surfaces, deteriorated septa, vent obstruction, or any other condition capable of compromising chamber integrity are repaired or replaced before sampling.

Chamber placement within each sampling unit should adequately represent the spatial variability of the soil surface under investigation. Under the standard LAPBio field configuration, collars are spatially distributed across the sampling area with an approximate separation of 3 m between adjacent chambers whenever site

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conditions permit. This distance is adopted as an operational guideline rather than as a rigid spatial grid. Chamber positions may therefore be adjusted according to the experimental design and local field conditions, including treatment boundaries, topographic gradients, crop rows, vegetation patches, microrelief, heterogeneous soil conditions, or other features relevant to the processes under investigation. Spatial replication is particularly important for soil GHG measurements because fluxes may exhibit substantial small-scale heterogeneity, directly affecting the representativeness and uncertainty of site-level estimates (CHARTERIS et al., 2020).

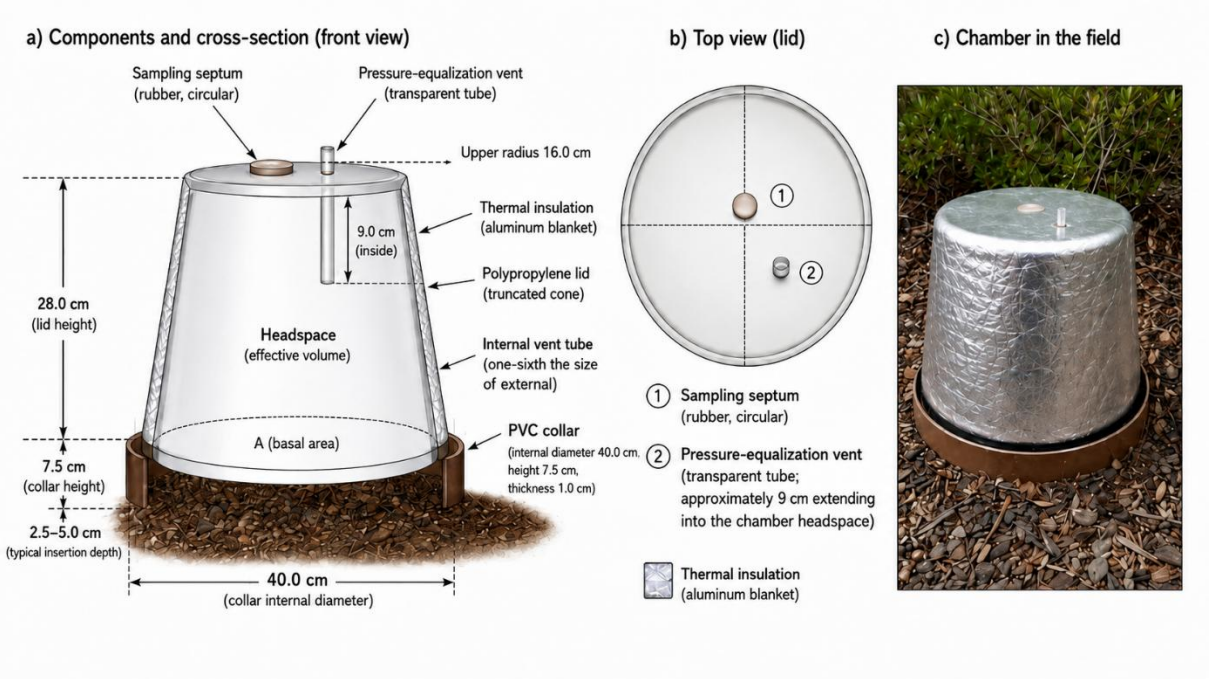

Figure 1. Static chamber system used by LAPBio for soil-atmosphere GHG flux measurements: (a) chamber cross-section and main dimensions; (b) lid configuration with sampling septum and pressure-equilibration vent; and (c) chamber deployed in the field. Source: Authors; schematic illustration enhanced with Gemini AI assistance.

Figura 1. Sistema de câmara estática utilizado pelo LAPBio para medições do fluxo de GEE no solo-atmosfera: (a) seção transversal e dimensões principais da câmara; (b) configuração da tampa com septo de amostragem e ventilação de equilíbrio de pressão; e (c) câmara instalada no campo. Fonte: Autores; ilustração esquemática aprimorada com assistência da IA Gemini.

The standard LAPBio protocol employs ten chambers per sampling unit, defined according to the experimental design, such as a plot, treatment, land-cover class, or study site. This replication is intended to improve representation of within-site spatial heterogeneity and should not be interpreted

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as a universal minimum number of chambers. Appropriate replication depends on spatial variability, the gas under investigation, expected flux magnitude, sampling frequency, and study objectives (CHARTERIS et al., 2020). Whenever fewer than ten chambers are used, the actual replication and spatial arrangement should be explicitly recorded and reported.

Collars are inserted approximately 2.5–5.0 cm into the soil, preferably on relatively level surfaces and with minimal disturbance of the surrounding area. Under the standard LAPBio protocol, collar installation is performed at least 24 h before the first gas sampling whenever field conditions permit. Chamber-base insertion may disturb soil structure, roots, and gas-transport pathways and can consequently induce short-term changes in measured gas exchange. Allowing an equilibration period after installation therefore reduces the influence of installation-induced disturbance on subsequent flux measurements (PAVELKA et al., 2018; CHARTERIS et al., 2020). Soil compaction and unnecessary displacement of litter, residues, or surface material within the chamber footprint should be avoided.

In agricultural systems where soil-derived fluxes are the principal target, collars are preferentially positioned between crop plants without enclosing aboveground plant structures. In pasture systems, vegetation may remain within the chamber footprint when it forms part of the soil–plant system under investigation. Vegetation management and chamber placement should remain consistent among replicates and sampling campaigns. Departures from the approximate 3-m spacing may be required when chamber locations must represent contrasting treatments, topographic positions, vegetation conditions, management rows, soil characteristics, or other predefined spatial units. In such cases, the arrangement should prioritize the experimental design and environmental representativeness rather than adherence to a fixed inter-chamber distance.

The geographic coordinates of the sampling area and, where appropriate, the positions of individual chambers are recorded. Each chamber receives a unique identification code that is maintained throughout field sampling, laboratory analysis, data processing, and flux calculation.

The effective chamber headspace is determined individually at each sampling event because collar insertion depth and irregularities in the soil surface alter the actual volume enclosed during chamber closure. The exposed collar height is therefore measured for each chamber and combined with chamber geometry and the enclosed basal area to calculate the effective internal volume. The basal area used for flux determination corresponds to the soil surface enclosed by the collar. Chamber-specific volume and area values are retained in the field database and subsequently incorporated into flux calculation.

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Gas sampling and environmental measurements

Gas sampling begins after the removable lid is fitted to the previously installed collar and the chamber is sealed. LAPBio routinely performs measurements between 10:00 and 12:00 local standard time (UTC–03:00), corresponding to 13:00–15:00 UTC, to maintain a consistent sampling window among campaigns and reduce variability associated with measurements conducted at markedly different times of day. This interval represents a standardized operational feature of the LAPBio protocol and should not be interpreted as universally representative of the daily mean flux, because diel patterns may vary among gases, ecosystems, soil conditions, vegetation types, and meteorological conditions (DAVIDSON et al., 2002; CHARTERIS et al., 2020). Representative components of the field sampling procedure are presented in figure 2.

Figure 2. Main components: (a) gas sampling from the static chamber; (b) transfer to a headspace vial using a syringe with three-way stopcock; (c) sequential samples stored in labeled vials for chromatographic analysis. Source: Authors.

Figura 2. Componentes principais: (a) amostragem de gás da câmara estática; (b) transferência para um frasco de headspace usando uma seringa com válvula de três vias; (c) amostras sequenciais armazenadas em frascos rotulados para análise cromatográfica. Fonte: Autores.

When ten chambers are used, deployment may be performed simultaneously, sequentially, or in two successive groups of five chambers, depending on field logistics and personnel availability. Regardless of the deployment strategy, the exact closure time of each chamber is recorded. Gas samples are collected at nominal elapsed times of 1, 10, 20, and 30 min after chamber closure. The actual sampling time for each chamber and vial is recorded and subsequently used to establish the concentration–time relationship. Thus, flux calculation is based on the effective elapsed time rather than on the nominal sampling schedule whenever operational differences occur.

Immediately before each gas withdrawal, the chamber headspace is homogenized for approximately 30 s using the same 60-mL syringe employed for sample collection. During this period, chamber air is repeatedly and gently drawn into and returned from the syringe through the sampling septum, producing a controlled pumping action intended to homogenize the gas within the headspace. Plunger movements are performed smoothly to avoid abrupt pressure changes or unnecessary disturbance of chamber conditions. After approximately 30 s of pumping, the gas sample is withdrawn for transfer to the storage vial. Standardization of this procedure among chambers and sampling times is important because manual sampling and headspace mixing can influence measured gas concentrations and consequently affect flux estimation (CHRISTIANSEN et al., 2011).

Gas is withdrawn through the sampling septum using a 60-mL polypropylene syringe equipped with a three-way stopcock, Luer Lock connection, and needle. Approximately 20–25 mL of chamber gas is collected at each sampling time and immediately transferred to a pre-evacuated 12-mL glass headspace vial. The transferred volume exceeds the internal volume of the vial, leaving the sample under slight positive pressure after injection. This procedure reduces the risk of ambient-air intrusion during storage and provides sufficient sample volume for subsequent chromatographic analysis (HARVEY et al., 2020). The volume withdrawn at each sampling time also represents substantially less than 1% of the chamber headspace, thereby limiting pressure and concentration disturbances associated with repeated sampling (COLLIER et al., 2014; HARVEY et al., 2020).

Vials are evacuated before field deployment and individually identified according to sampling unit, chamber number, date, and elapsed closure time. During sample transfer, the syringe, stopcock, needle, septum, and vial connection are handled carefully to avoid sample loss, contamination, or accidental depressurization. Sample identification is checked immediately after each transfer to preserve correspondence between field records and laboratory analyses.

At least one atmospheric background sample is collected for each sampling unit,

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preferably between the first and second chamber-sampling times. This sample provides a reference for ambient CO_2, CH_4, and N_2O concentrations at the study site and is subsequently used during quality-control assessment of the initial chamber concentrations. The atmospheric sample is not routinely used as a substitute for a chamber observation in the concentration–time sequence; its primary function is to characterize ambient conditions and assist in identifying anomalous initial concentrations.

Environmental and chamber variables required for flux calculation and interpretation are recorded during each sampling sequence. These include atmospheric pressure, air temperature, soil temperature, chamber-headspace temperature, and exposed collar height. Under the standard LAPBio protocol, atmospheric pressure, air temperature, and soil temperature are recorded once during each sampling sequence, between the first and second nominal gas-sampling times, corresponding approximately to the interval between 1 and 10 min after chamber closure. These measurements are intended to characterize the environmental conditions prevailing during the closure period. Chamber-headspace temperature, in turn, is measured individually for each chamber immediately after collecting the final gas sample, at approximately 30 min after closure. When repeated or continuous measurements of chamber-headspace temperature are available, the mean temperature during chamber closure is retained for flux calculation. The exposed collar height is measured individually for each chamber and used to determine the effective headspace volume.

Additional environmental variables may be recorded according to the objectives of each study. Soil water content, water-filled pore space (WFPS), recent precipitation, soil chemical properties, and other ancillary measurements may be particularly useful for interpreting temporal and spatial variability in soil GHG exchange. These variables are considered complementary to the physical parameters directly required for flux calculation and should be documented together with the corresponding sampling event.

A standardized field sheet is used to record sampling-unit identification, geographic coordinates, chamber and vial codes, personnel involved, chamber closure times, actual gas-sampling times, atmospheric pressure, air and soil temperatures, chamber-headspace temperature, exposed collar height, and any unusual field conditions observed during measurement. The timing of the environmental measurements follows the standardized procedure described above, allowing each record to be associated with the corresponding sampling sequence. Particular attention is given to events that may affect chamber performance or sample integrity, including inadequate sealing, accidental chamber displacement, septum damage, vent obstruction,

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water entry, sampling delays, or other operational anomalies. These records remain linked to the chromatographic dataset and are subsequently considered during quality-assurance and flux-validation procedures.

Rainfall and other environmental conditions occurring during sampling are also recorded in the field sheet. Sampling is interrupted only when precipitation compromises chamber sealing, sample integrity, operational safety, or completion of the prescribed sampling sequence. Rainfall itself is not considered an automatic criterion for data exclusion because changes in soil water conditions are intrinsically associated with the biogeochemical processes regulating CO_2, CH_4, and N_2O production and consumption. Any interrupted sequence is repeated only when field conditions permit completion of a technically valid measurement.

After collection, gas samples are protected from direct solar radiation and excessive heating. Under tropical field conditions, vials are transported in insulated containers under stable temperature conditions until laboratory analysis. Large temperature fluctuations and unnecessarily prolonged storage are avoided because appropriate collection, pressurization, storage, and handling are required to preserve the integrity of discrete gas samples intended for chromatographic determination (HARVEY et al., 2020). Samples are transported to the laboratory together with the corresponding field records, preserving complete traceability from chamber deployment to chromatographic analysis and subsequent flux calculation. The main operational steps of the LAPBio static-chamber protocol are summarized in box 1.

Gas Chromatographic Analysis

The concentrations of CO_2, CH_4, and N_2O in discrete gas samples collected in the field are determined by gas chromatography at LAPBio. The analytical system is configured to quantify all three gases within a single sequence, combining selective chromatographic separation with flame ionization and electron capture detection. Sample identification, chromatographic files, calibration records, and analytical results are maintained to ensure traceability. Procedures for calibration, sample handling, and instrument verification are critical for chamber-based measurements, as uncertainties introduced during laboratory analysis are subsequently propagated into soil–atmosphere flux estimates (COLLIER et al., 2014; HARVEY et al., 2020).

Chromatographic System and Analytical Conditions

Gas analyses are conducted using a gas chromatograph (TRACE 1310, Thermo Scientific, Waltham, MA, USA) configured for CO_2, CH_4, and N_2O determination. Carbon dioxide and CH_4 are separated on a HayeSep Q packed column and quantified with a flame ionization detector (FID), with a methanizer upstream of the FID for CO_2 conversion. Nitrous oxide is separated on a HayeSep

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D packed column and quantified using an electron capture detector (ECD).

Box 1. LAPBio field protocol for static-chamber GHG measurements.

Quadro 1. Protocolo de campo LAPBio para medições de GEE em câmara estática.

Stage	*Standard procedure*
Chamber preparation	Inspect collars, lids, septa, seals, venting system, and overall chamber integrity before field deployment.
Collar installation	Insert collars approximately 2.5–5.0 cm into the soil at least 24 h before the first gas sampling whenever field conditions permit, minimizing disturbance of soil, litter, and vegetation.
Spatial arrangement and replication	Use ten chambers per sampling unit under the standard LAPBio protocol, with an approximate 3-m separation between adjacent chambers when site conditions permit. Adjust chamber positions according to experimental design and local spatial heterogeneity and record any deviation from the standard arrangement.
Chamber closure	Fit and seal the lid within the standardized sampling window and record the exact closure time of each chamber.
Headspace homogenization	Immediately before each gas withdrawal, homogenize the chamber headspace for approximately 30 s by gently drawing chamber air into and returning it from the 60-mL syringe through the sampling septum.
Gas collection	Collect chamber gas at nominal elapsed times of 1, 10, 20, and 30 min after closure, recording the actual sampling time for each sample.
Sample transfer	After headspace homogenization, withdraw approximately 20–25 mL of chamber gas using a 60-mL polypropylene syringe equipped with a three-way stopcock and transfer it immediately to a pre-evacuated 12-mL glass vial.
Atmospheric reference	Collect at least one ambient-air sample for each sampling unit, preferably between the first and second chamber-sampling times.
Environmental measurements	Record atmospheric pressure and air and soil temperatures between the first and second gas-sampling times. Measure chamber-headspace temperature immediately after the final gas sample and record exposed collar height for determination of effective headspace volume.
Field observations	Record rainfall, sealing problems, sampling delays, chamber disturbance, vent obstruction, water entry, or any other condition that may affect chamber performance or sample integrity.
Sample storage and transport	Protect vials from direct solar radiation and excessive heating and transport them to the laboratory in insulated containers under stable temperature conditions.

Source: Authors. *Fonte: autores*

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Analytical-grade helium (grade 5.0) is used as the carrier gas at a flow rate of 20 mL min^{-1}. The chromatographic oven is maintained at 60 °C throughout the 6-min analytical run. The auxiliary heated zones associated with the valve oven are maintained at 60 °C (Heater 3) and 350 °C (Heater 4). The FID operates at 250 °C, the ECD at 350 °C, and the methanizer at 280 °C. The chromatographic system contains two 1-mL sampling loops associated with the respective analytical lines. Gas samples are introduced manually, whereas chromatographic separation, detector-signal acquisition, and data recording proceed automatically according to the established analytical method. Including sample introduction and system operation, a complete analytical cycle requires approximately 7 min per sample.

Before an analytical sequence begins, the chromatographic system is allowed to reach stable operating conditions. Samples are analyzed according to their field identification codes, preserving the correspondence among sampling unit, chamber, closure time, vial, and chromatographic record. Field samples, atmospheric reference samples, and calibration standards are processed within the same analytical workflow.

Calibration and Analytical Performance

Gas concentrations are determined using multipoint calibration curves established for CO_2, CH_4, and N_2O. The calibration system includes a primary reference gas standard supplied by White Martins/Linde (PA), working gas standards prepared at LAPBio by dilution of PA with analytical-grade nitrogen (PA1, PA3, and PA4), and an additional certified gas standard supplied by Glacier Energy (PB). Together, these standards provide calibration points covering the concentration ranges required for the analysis of atmospheric and chamber-headspace samples.

The PA-derived working standards were prepared at LAPBio by dilution of the original multicomponent PA gas mixture with analytical-grade N_2. Following preparation, the mixing ratios of CO_2, CH_4, and N_2O in each working standard were experimentally determined. These assigned mixing ratios, rather than theoretical concentrations calculated solely from the dilution procedure, are used for chromatographic calibration. Each standard is individually identified, and its assigned gas mixing ratios are maintained in the laboratory calibration records to ensure analytical traceability.

The standard gas mixing ratios adopted for calibration are presented in box 2. CO_2 is expressed as parts per million by volume (ppmv), whereas CH_4 and N_2O are expressed as parts per billion by volume (ppbv).

For each analyte, the assigned gas mixing ratio (x) is related by linear regression to the corresponding chromatographic detector response (y), expressed as integrated peak area. The resulting calibration function is used to convert detector

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responses from field samples into CO_2, CH_4, and N_2O mixing ratios. Calibration performance is evaluated from the regression parameters, coefficient of determination, consistency of detector response across the working range, and inspection of chromatographic behavior. The chromatographic system allows us to reach stable operating conditions before calibration and sample analysis, and its response is verified during routine analytical sequences to identify possible instrumental drift or instability.

Box 2. Standard gas ratios for chromatographic calibration of CO_2, CH_4, and N_2O at LAPBio.

Quadro 2. Razões de mistura dos gases padrão utilizados na calibração cromatográfica de CO_2, CH_4 e N_2O em LAPBio.

Standard	Origin / Preparation	CO_2 (ppmv)	CH_4 (ppbv)	N_2O (ppbv)
PA1	Working standard prepared at LAPBio (from PA and analytical-grade N_2)	205.7	953.6	243.4
PA4	Working standard prepared at LAPBio (from PA and analytical-grade N_2)	309.2	1399.3	350.5
PB	Certified gas standard supplied by Glacier Energy (USA)	385.3	1835.3	322.0
PA3	Working standard prepared at LAPBio (from PA and analytical-grade N_2)	563.1	2541.3	613.1
PA	Primary reference gas standard supplied by White Martins/Linde (Brazil)	703.5	3052.0	763.4

Source: Authors /*Fonte: Autores*

A representative calibration showed highly linear detector responses for all three gases across the working concentration ranges (figure 3). For CO_2, the relationship between mixing ratio and integrated peak area was described by ($y = 0.17576x + 3.79476$), with ($R2 = 0.9948$). For CH_4, the fitted relationship was ($y = 0.00017x + 0.00869$), with ($R2 = 1.0000$). For N_2O, the corresponding relationship was ($y = 0.00005x + 0.00042$), with ($R2 = 0.9982$). These coefficients demonstrate a highly linear instrumental response across the concentration ranges represented by the calibration standards.

Under the analytical conditions adopted by LAPBio, analytical precision is better than 1% for CO_2 and CH_4 and better than 2% for N_2O. Calibration records and chromatograms are retained as part of the analytical database.

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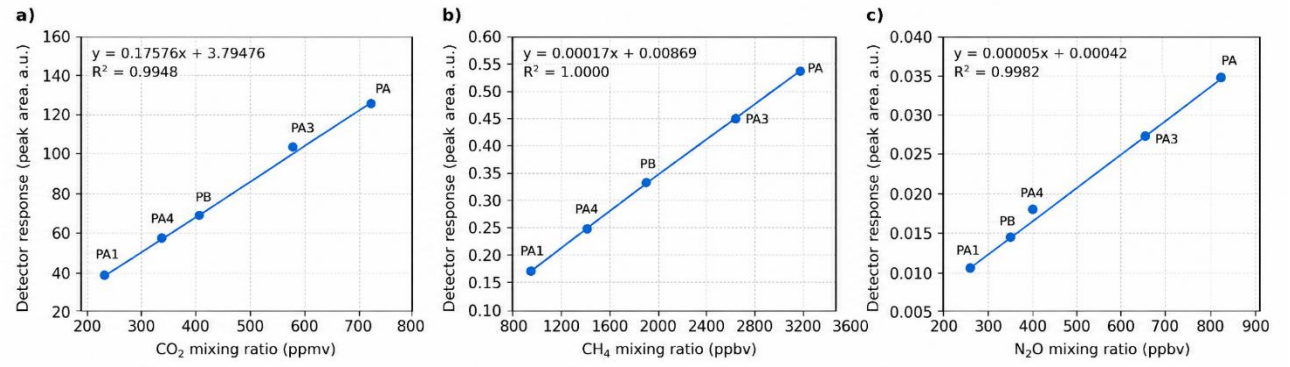

Figure 3. Representative calibration curves for chromatographic determination of (a) CO_2 by FID after methanization, (b) CH_4 by FID, and (c) N_2O by ECD. Symbols represent the gas standards adopted by LAPBio, and solid lines indicate the fitted linear regressions. Source: Authors.

Figura 3. Curvas de calibração representativas para a determinação cromatográfica de (a) CO por FID após metanização, (b) CH por FID e (c) N O por ECD. Símbolos representam os padrões de gás adotados pelo LAPBio, e linhas sólidas indicam as regressões lineares ajustadas. Fonte: Autores.

Samples presenting anomalous detector responses, unexpected peak characteristics, or inconsistencies with the temporal concentration sequence of the corresponding chamber are individually examined and subsequently evaluated according to quality-assurance and flux-validation procedures.

Flux Calculation and Quality Control

Gas fluxes are calculated from the temporal change in CO_2, CH_4, or N_2O mixing ratio within the chamber headspace during the closure period. Flux determination integrates the measured concentration–time response with chamber-specific geometric parameters and the pressure and temperature conditions recorded during sampling. Because chamber deployment can modify the concentration gradient between soil and atmosphere, flux estimation requires consistent calculation procedures and explicit quality-control criteria (VENTEREA, 2010; VENTEREA et al., 2020). The LAPBio procedure therefore combines a standardized flux equation with individual assessment of each chamber concentration sequence before the resulting flux is accepted for subsequent analysis.

Flux Calculation

For each chamber deployment, the four gas concentrations measured at 1, 10, 20, and 30 min are associated with the actual elapsed sampling times recorded in the field. The rate of change in gas mixing ratio ($d\chi/dt$) is initially estimated by ordinary least-squares linear regression of gas mixing ratio

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against elapsed time. Actual sampling times, rather than nominal collection times, are used in the regression whenever small operational differences occur during field sampling.

The soil–atmosphere gas flux is calculated according to Eq. (1):

$$F = \frac{d\chi}{dt} \; x \; \frac{V}{A} \; x \; \frac{PM}{RT} \qquad \text{(Eq. 1)}$$

where:

- F is the soil–atmosphere gas flux;
- dχ/dt is the rate of change in gas mixing ratio within the chamber headspace;
- V is the effective chamber headspace volume (L);
- A is the projected soil surface area enclosed by the chamber (m^2);
- P is atmospheric pressure (atm);
- R is the universal gas constant (0.082057 L atm mol^{-1} K^{-1});
- T is the absolute chamber-headspace temperature (K), measured inside the chamber immediately after collection of the final gas sample, at approximately 30 min after closure. When repeated or continuous headspace-temperature measurements are available, the mean temperature during chamber closure is used; and
- M is the molar mass of the gas (g mol^{-1}).

Equation (1), derived from the ideal gas law, converts the temporal change in gas mixing ratio into a mass flux per unit soil surface area. When dχ/dt is expressed as mol mol^{-1} h^{-1}, the equation yields flux in g m^{-2} h^{-1}. Chromatographically determined gas mixing ratios are therefore converted to molar fractions before flux calculation. For CO_2, slopes originally expressed in ppmv min^{-1} are multiplied by 10^{-6} and by 60 to obtain mol mol^{-1} h^{-1}. For CH_4 and N_2O, slopes expressed in ppbv min^{-1} are multiplied by 10^{-9} and by 60.

For the standard LAPBio chamber, the projected basal area is calculated from the 40.0-cm internal collar diameter and corresponds to approximately 0.1257 m^2. In contrast, the effective headspace volume is determined individually for each chamber because it depends on the exposed collar height measured during sampling. Atmospheric pressure and chamber-headspace temperature correspond to the conditions recorded during the respective field deployment. When only a single chamber-headspace temperature measurement is available, the value recorded immediately after collection of the final gas sample, at approximately 30 min after chamber closure, is used. Chamber-headspace temperature is measured by inserting a temperature probe into the chamber headspace through the designated measurement access, avoiding contact with the chamber walls or soil surface. When repeated or continuous measurements are available, the mean chamber-headspace temperature during the closure period is

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preferred for flux calculation.

Molar masses of 44.01 g mol^{-1} for CO_2, 16.04 g mol^{-1} for CH_4, and 44.01 g mol^{-1} for N_2O are used. Final CO_2 fluxes are expressed as mg CO_2 m^{-2} h^{-1}, whereas CH_4 and N_2O fluxes are expressed as µg CH_4 m^{-2} h^{-1} and µg N_2O m^{-2} h^{-1}, respectively. Positive fluxes indicate net transfer from the soil–chamber system to the atmosphere, whereas negative fluxes indicate net uptake or consumption by the soil–ecosystem system.

For each fitted regression, LAPBio records the slope, intercept, coefficient of determination (R^2), F-statistic, and associated probability value (p). These parameters are retained together with the individual concentration measurements and are subsequently considered during flux validation.

Linear regression is adopted as the standard LAPBio flux-calculation procedure because routine chamber deployment comprises four discrete observations collected during a 30-min closure period. Nevertheless, chamber closure may induce departures from linear concentration accumulation, particularly when changes in headspace concentration progressively alter the soil–atmosphere concentration gradient. Linear and nonlinear calculation approaches may therefore yield different flux estimates, with trade-offs between accuracy and precision (Venterea, 2010; Venterea et al., 2020). When a concentration sequence presents consistent evidence of curvature that cannot be attributed to field or analytical error, an alternative flux-calculation approach may be evaluated. Any departure from the standard linear procedure must be explicitly documented in the corresponding study.

Quality Assurance and Flux Validation

Quality control is applied to each chamber deployment before fluxes are incorporated into the final dataset. Evaluation considers the complete concentration–time sequence, chromatographic performance, atmospheric reference concentration, field records, regression behavior, and concentration change relative to analytical variability and the gas-specific minimum detectable flux (MDF). Linear regressions are assessed using the coefficient of determination (R^2) and the F-test from regression analysis of variance. No single statistical criterion is used as the sole basis for accepting or rejecting a flux.

Under the standard LAPBio procedure, $R^2 \geq 0.90$ is considered an operational indicator of a well-defined linear concentration–time response. Regression significance is additionally evaluated using the F-test at $\alpha = 0.05$, testing the null hypothesis that the concentration–time slope equals zero. A high R^2 combined with a significant F-test provides strong evidence of a detectable temporal trend during chamber closure. These statistics are used as screening and diagnostic criteria rather than absolute exclusion thresholds. Sequences with $R^2 < 0.90$ or a non-significant F-test

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are individually examined, particularly when the estimated flux is small or approaches the gas-specific MDF. Automatic exclusion based solely on regression statistics could preferentially remove low or near-zero fluxes and introduce bias (PARKIN et al., 2012; VENTEREA et al., 2020).

The initial chamber concentration is compared with the atmospheric background sample collected for the corresponding sampling unit. Reasonable agreement provides additional evidence of consistent chamber closure, sample identification, and gas transfer. However, differences between the first chamber concentration and the atmospheric reference are not automatic rejection criteria, because spatial heterogeneity and gas accumulation immediately after closure may contribute to such discrepancies. Marked differences trigger inspection of the concentration sequence, chromatogram, sampling record, and field observations.

Positive and negative concentration slopes are both physically admissible. Positive slopes indicate net gas emission, whereas negative slopes indicate net uptake or consumption. Negative fluxes are retained whenever the concentration–time sequence is analytically consistent and no field or chromatographic anomaly is identified. Likewise, low or near-zero fluxes are not discarded solely because they exhibit weak regression statistics. Gas-specific minimum detectable fluxes (MDFs) characterize the lower measurement capability of the chamber–chromatography system.

At LAPBio, MDFs were estimated from the experimentally determined analytical limit of detection (LOD) and the characteristics of the static-chamber deployment, following Parkin et al. (2012). Under the current LAPBio configuration, the estimated MDFs are 90.8 mg CO_2 m^{-2} h^{-1} for CO_2, 32.0 µg CH_4 m^{-2} h^{-1} for CH_4, and 54.5 µg N_2O m^{-2} h^{-1} for N_2O. Fluxes below the corresponding MDF are retained in the analytical database but interpreted as not quantitatively distinguishable from the lower detection capability of the system. The analytical LOD expressed as gas concentration is therefore distinguished from the flux detection limit, which also depends on chamber geometry, deployment duration, sampling frequency, and analytical variability (PARKIN et al., 2012).

Coherent nonlinear concentration–time responses are flagged for individual evaluation rather than automatically rejected. Alternative nonlinear or diffusion-based approaches may be applied when justified by chamber configuration, sampling intensity, soil conditions, and analytical precision (VENTEREA, 2010; VENTEREA et al., 2020). Any departure from the standard LAPBio linear procedure must be documented. Flux exclusion is restricted to measurements compromised by documented technical failure, including leakage, sealing problems, chamber displacement, vent obstruction, sample misidentification, contamination, loss of sample integrity,

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chromatographic failure, uncorrectable peak integration, or insufficient valid observations. Corrections and exclusions are retained in the analytical record together with their justification.

For operational purposes, individual chamber sequences are classified as accepted, accepted after review, or rejected, as summarized in box 3. Field observations, chromatographic records, regression parameters, MDF classification, corrections, and exclusions are maintained in the analytical database, allowing reconstruction of the complete measurement and validation process.

Box 3. Quality-control classification for static-chamber flux measurements. Source: Authors.

Quadro 3. Classificação de controle de qualidade para medições de fluxo em câmara estática. Fonte: Autores.

Classification	*Main criteria*	*Action*
Accepted	Complete concentration sequence; coherent temporal response; no identified field or analytical anomaly; regression statistics consistent with the magnitude of the observed concentration change.	Flux retained
Accepted after review	$R^2 < 0.90$; non-significant F-test; flux near or below the gas-specific MDF; initial concentration differing markedly from the atmospheric reference; possible curvature; or isolated analytical irregularity without evidence of failure	Concentration sequence, regression statistics, MDF, chromatograms, and field records are reviewed; flux retained when technically defensible and appropriately flagged
Rejected	Documented leakage, sealing failure, chamber disturbance, vent obstruction, sample misidentification, contamination, chromatographic failure, compromised sample integrity, or insufficient valid observations for flux calculation	Flux excluded and reason documented

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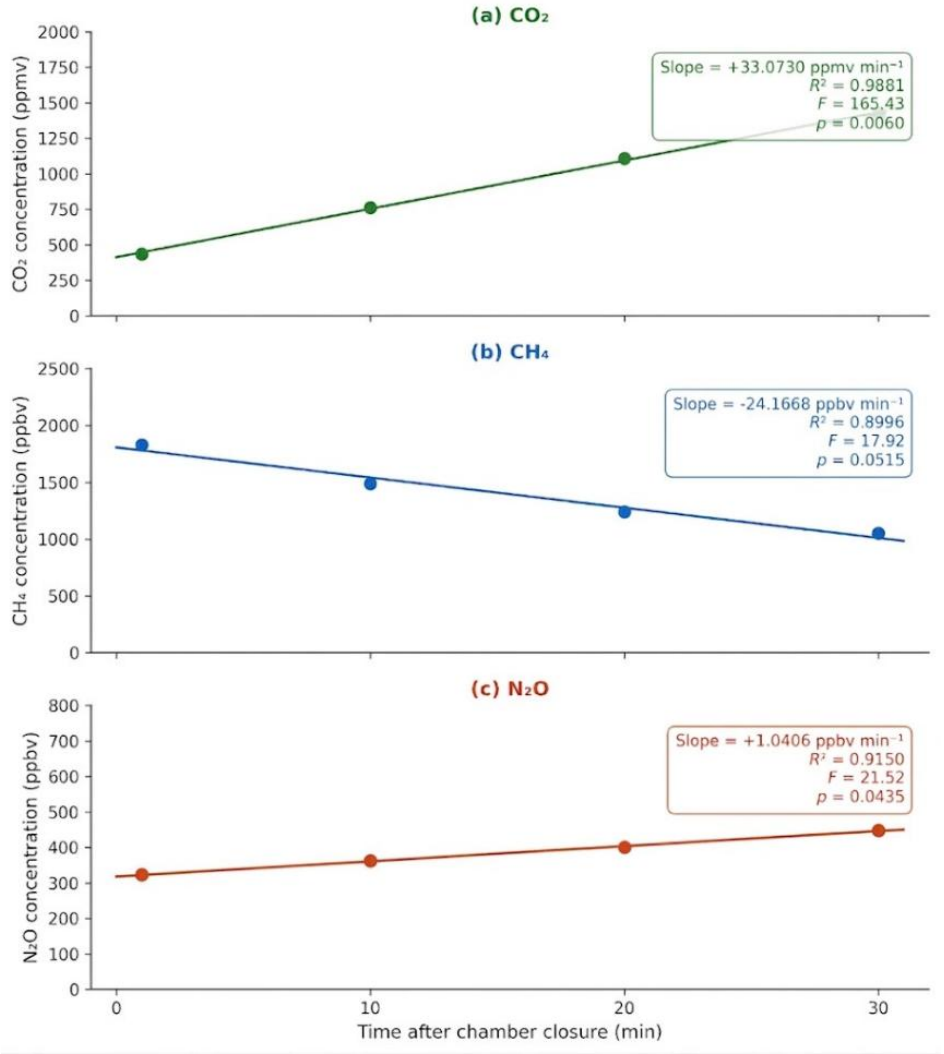

Figure 4. Concentration–time relationships for (a) CO_2, (b) CH_4, and (c) N_2O during a 30-min chamber deployment. Symbols represent the four gas samples and solid lines the ordinary least-squares fits. Slope, R^2, F, and *p* are shown for each gas.

Figura 4. Relações concentração-tempo para (a) CO, (b) CH e (c) N O durante uma implantação de câmara de 30 minutos. Os símbolos representam as quatro amostras de gás e as linhas sólidas, os ajustes por mínimos quadrados ordinários. Inclinação, R^2, F e p são mostrados para cada gás.

Method Application

To demonstrate the complete analytical workflow described in this Technical Note, a representative chamber deployment from a LAPBio field dataset was reprocessed following the procedures described above. The example is

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intended solely to illustrate methodological implementation, from concentration–time fitting to flux calculation and quality-control classification, without supporting ecological inference. A single deployment was selected for the simultaneous evaluation of CO_2, CH_4, and N_2O under the same chamber geometry and environmental conditions.

Four gas samples collected at 1, 10, 20, and 30 min after chamber closure were included in ordinary least-squares regressions. CO_2 mixing ratios were expressed in ppmv, whereas CH_4 and N_2O were converted to ppbv. All observations were retained in the initial fits, regardless of whether removing an individual point could increase R^2. This differs from earlier exploratory routines in which three-point subsets could be selected according to the highest R^2; under the present protocol, any exclusion must be supported by documented field or analytical evidence.

For the selected deployment, CO_2 increased from 435.09 to 1422.09 ppmv, yielding a slope of +33.0730 ppmv min^{-1} (R^2 = 0.9881; F = 165.43; p = 0.0060). CH_4 decreased from 1830.38 to 1050.28 ppbv, with a slope of −24.1668 ppbv min^{-1} (R^2 = 0.8996; F = 17.92; p = 0.0515), whereas N_2O increased from 383.31 to 411.56 ppbv, yielding +1.0406 ppbv min^{-1} (R^2 = 0.9150; F = 21.52; p = 0.0435). These concentration–time relationships are shown in figure 4.

The recorded total chamber height was 33 cm. Considering the 28-cm polypropylene lid, the exposed collar height was 5.0 cm. Combining the approximately 27.8-L lid volume with the exposed collar volume resulted in an effective headspace of approximately 34.09 L and a V/A ratio of 0.2713 m. Atmospheric pressure was 0.92445 atm, and chamber-headspace temperature was 23.6 °C (296.8 K).

Application of Eq. (1) yielded a CO_2 flux of +899.4 mg CO_2 m^{-2} h^{-1}, a CH_4 flux of −239.5 µg CH_4 m^{-2} h^{-1}, and an N_2O flux of +28.3 µg N_2O m^{-2} h^{-1}. The sign convention established in the Flux Calculation section was preserved, such that the positive CO_2 and N_2O fluxes indicate net soil-to-atmosphere transfer, whereas the negative CH_4 flux indicates net uptake by the soil. The regression diagnostics, calculated fluxes, and corresponding MDF evaluation are summarized in table 1.

The three gases illustrate complementary aspects of the validation procedure. The CO_2 sequence satisfies the operational regression criteria and produces a flux substantially above its MDF, supporting its direct acceptance. The CH_4 sequence presents a coherent negative concentration trend and a flux magnitude well above the CH_4 MDF, although R^2 is marginally below 0.90 and the F-test is marginally non-significant at $\alpha = 0.05$. Rather than being automatically rejected, this measurement is therefore classified as accepted after review, illustrating the importance of interpreting regression diagnostics together with flux magnitude and analytical evidence.

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Table 1. Regression diagnostics, calculated fluxes, and quality-control classification for the illustrative chamber deployment. Source: Authors.

Tabela 1. Diagnósticos de regressão, fluxos calculados e classificação de controle de qualidade para a implantação ilustrativa da câmara. Fonte: Autores

Gas	Slope	R^2	F	p	Calculated flux	MDF	QA/QC interpretation
CO_2	+33.0730 ppmv min^{-1}	0.9881	165.43	0.0060	+899.4 mg m^{-2} h^{-1}	90.8 mg m^{-2} h^{-1}	Accepted
CH_4	−24.1668 ppbv min^{-1}	0.8996	17.92	0.0515	−239.5 µg m^{-2} h^{-1}	32.0 µg m^{-2} h^{-1}	Accepted after review
N_2O	+1.0406 ppbv min^{-1}	0.9150	21.52	0.0435	+28.3 µg m^{-2} h^{-1}	54.5 µg m^{-2} h^{-1}	Accepted after review; below MDF

The N_2O sequence provides a different example. Its regression satisfies both the operational R^2 criterion and the F-test criterion; however, the calculated flux of +28.3 µg N_2O m^{-2} h^{-1} is below the corresponding MDF of 54.5 µg N_2O m^{-2} h^{-1}. The observation is therefore retained in the analytical database but flagged as below the measurement detection capability and should not be interpreted as a quantitatively distinguishable net N_2O flux. This case illustrates why satisfactory regression statistics alone do not establish flux detectability.

Taken together, the illustrative application demonstrates the sequential nature of the LAPBio protocol: complete concentration–time series are first fitted and statistically evaluated; technically defensible slopes are converted to fluxes using chamber-specific geometry and environmental conditions; the resulting estimates are compared with gas-specific MDFs; and final classification integrates regression statistics, analytical performance, field information, and measurement detectability. This framework preserves environmentally plausible low and negative fluxes while restricting exclusion to observations for which technical measurement failure can be documented.

FINAL REMARKS

The protocol described in this Technical Note establishes a standardized and traceable procedure for determining soil–atmosphere CO_2, CH_4, and N_2O fluxes using non-flow-through, non-steady-state static chambers. Its application integrates field deployment, discrete gas sampling, chromatographic analysis, chamber-specific flux calculation, and quality-control procedures within a

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single methodological framework.

Particular emphasis is placed on the use of actual sampling times, chamber-specific headspace volume, measured pressure and headspace temperature, multipoint chromatographic calibration, and individual evaluation of each concentration–time sequence. Regression statistics are treated as diagnostic criteria rather than automatic exclusion rules, while gas-specific minimum detectable fluxes provide an additional basis for interpreting low-magnitude measurements.

The protocol also prioritizes traceability. Field observations, chromatographic records, regression parameters, analytical corrections, MDF classification, and exclusion decisions should remain linked throughout data processing. This approach preserves technically defensible low and negative fluxes while restricting data exclusion to cases supported by documented field or analytical failure.

Although the procedures presented here define the standard workflow adopted by LAPBio, chamber-based measurements remain sensitive to site conditions, experimental design, and the temporal dynamics of soil greenhouse gas exchange. Adjustments in replication, sampling frequency, closure duration, or flux-model formulation may therefore be required for specific applications, provided that such modifications are explicitly documented. The protocol thus provides a reproducible methodological reference for future studies of soil greenhouse gas fluxes in tropical environments.

Declaration on the use of Generative AI

Generative artificial intelligence was used only as an auxiliary tool for language editing, text organization, and visual refinement. The authors independently reviewed and validated all scientific content, methodological procedures, calculations, interpretations, and final manuscript decisions.

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