Sep 23, 2024

USDA LTAR Common Experiment Measurement: Chamber-based fluxes of nitrous oxide (N_2O) and methane (CH_4) from soil

DOI
dx.doi.org/10.17504/protocols.io.yxmvmemw5g3p/v1

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DOI: dx.doi.org/10.17504/protocols.io.yxmvmemw5g3p/v1

External link: https://ltar.ars.usda.gov

Protocol Citation: G. Philip Robertson, Michel A. Cavigelli, Harold P. Collins, Curtis Dell, David R. Huggins, Virginia L. Jin, Kevin Kahmark, Mark A. Liebig 2024. USDA LTAR Common Experiment Measurement: Chamber-based fluxes of nitrous oxide (N2O) and methane (CH4) from soil. **protocols.io https://dx.doi.org/10.17504/protocols.io.yxmvmemw5g3p/v1**

Protocol status: Working
We use this protocol and it's working

Created: August 13, 2024

Last Modified: September 23, 2024

Protocol Integer ID: 105684

Keywords: Long-Term Agroecosystem Research, LTAR, USDA LTAR, Common Experiment, crops, nitrous oxide, N2O, methane, CH4, greenhouse gas, chambers, flux, cropland, grazing land

Funders Acknowledgement:
United States Department of Agriculture
Grant ID: -
NSF Long-Term Ecological Research Program
Grant ID: DEB 2224712
Michigan State University AgBioResearch
Grant ID: -

Disclaimer

This research is a contribution from the Long-Term Agroecosystem Research (LTAR) network. LTAR is supported by the United States Department of Agriculture. Support for this research was also provided by the NSF Long-Term Ecological Research Program (DEB 2224712) at the Kellogg Biological Station and by Michigan State University AgBioResearch.

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Abstract

Emissions of nitrous oxide (N_2O) and methane (CH_4) from soil are dynamic and spatially variable. Chamber based methods have been used since the 1980s to document fluxes and are widely used in both managed and natural ecosystems (Clough et al., 2020; Holland et al., 1999; Parkin and Venterea, 2010). They are well-tested, relatively inexpensive, and can be deployed extensively. They are also suitable for emissions of other gases, notably CO_2 and NO_x. Chambers can also be automated (e.g, Grace et al., 2020) to capture temporal dynamics. Here we provide hands-on, best-practice guidelines for the minimum and preferred chamber sampling plans sufficient to compare Common Experiment flux differences across all sites in the Long-Term Agroecosystem Research (LTAR) Network. Although each site is different, and exceptions will likely be necessary, following these guidelines will maximize the potential for making cross-site comparisons and provides those new to soil greenhouse gas sampling a straightforward path for successful measurement campaigns.

In general, chambers will need to be of a sufficient size and placed to capture representative fluxes, keeping in mind plant spacing, fertilizer placement, and other features of the site that affect spatial variability such as topography. Once placed, chambers will need to be sampled at intervals that capture episodic emissions that result from management events such as tillage and nitrogen fertilization and environmental events such as rainfall following dry periods. Fluxes are typically low during dry periods and in winter if freeze-thaw events are infrequent, and during these periods sampling frequencies can be relaxed. In general, we recommend a minimum of weekly sampling following events known to stimulate fluxes (tillage, N fertilization, rainfall following drought) and biweekly sampling otherwise except when soil is very dry or frozen, when sampling can be monthly. By convention, fluxes should be expressed as μg N_2O-N m^{-2} h^{-1} or μg CH_4-C m^{-2} h^{-1}.

Guidelines

General Principles

Agriculture in general and agricultural soils in particular are responsible for much of the contemporary increase in atmospheric N_2O, now increasing at the rate of 2% per decade (Tian et al., 2020). Understanding ecosystem-level controls on soil-borne fluxes and defining opportunities to mitigate emissions is fundamental to lowering atmospheric loading rates. Likewise, atmospheric CH_4 concentrations are increasing at an alarming rate, and while agricultural soils – excepting paddy rice – do not contribute significantly to this increase (Robertson, 2004), soil CH_4 oxidation (methanotroph consumption) represents a potential source of negative emissions, particularly in semiarid grazing lands and perennial herbaceous crops such as switchgrass (e.g., Bates et al., 2021; Liebig et al., 2012). Documenting the impact of different agricultural management strategies on emissions of both N_2O and CH_4 is important for evaluating the environmental performance of alternative production systems, for developing novel mitigation opportunities, and for validating simulation models that can be used to estimate global impacts of management change.

Chamber-based methods for capturing soil greenhouse gas emissions have been used for decades and are the preferred method for capturing flux differences among different experimental treatments and, when sampled frequently, can be used to estimate annual fluxes (see Clough et al., 2020; Holland et al., 1999; Parkin and Venterea, 2010). Although eddy covariance methods that continuously sample large fetches are superior in many respects, they are expensive and, for N_2O, sensors are currently limited to closed-path designs that require line power for precision pumps. Open-path N_2O sensors, which require less power, are under development (Pan et al., 2022) but not yet commercially available. Eddy covariance methods are also not well-suited for plot-based research because of their need for large fetch areas.

Sampling to detect differences in emissions among management systems such as those in the LTAR Common Experiment (CE) (Spiegal et al., 2018; Liebig et al., 2024) requires the deployment of appropriately sized chambers sampled at intervals sufficient to capture these differences. Because Common Experiment treatments typically differ in major ways with respect to crops and crop rotations; nitrogen fertilizer rate, timing, placement, and formulation; manure use; tillage and cultivation practices; cover crops; grazing and fire management; and other factors that can affect greenhouse gas emissions, it will be important to sample all treatments even when only one may be emitting detectable quantities. Moreover, to maximize statistical power for detecting treatment differences, it is more important to sample all replicate plots or fields rather than to sample multiple chambers in fewer plots or fields (Kravchenko and Robertson, 2015).

Chamber design

Chambers consist of two parts – a base that is inserted into the soil a few cm and left in place between field operations, and a lid that can be sealed to the base to provide a gas tight seal between the chamber headspace and the atmosphere. A pigtail vent in the lid or side of the chamber keeps atmospheric pressure changes from affecting flux estimates. The size of the chamber is important – usually 30 cm diameter or square is sufficient, and a height that does not preclude plants where present and does not overly dilute detectable headspace gas concentrations, e.g., 20 cm (Clough et al., 2020; Kahmark et al., 2020). The lid is left in place for up to an hour, and during this period the rate of accumulation of gas in the

headspace represents the soil gas flux. In most agricultural soils the net flux is generally positive for N_2O and negative for CH_4.

Analysis methods

Two analysis methods for quantifying soil gas flux are available. For the first, called the syringe method, a several mL headspace sample is removed through a rubber septum (installed in the chamber lid) by a needle and syringe and transferred to a pre-labeled gas-tight vial that is then taken to the lab for gas chromatograph analysis; at least four samples need to be taken over the chamber closure period. For the second, called the flow-through method, a portable flow-through analyzer is connected by tubing to inlet and outlet ports on the lid to circulate headspace gas past a sensor that reports gas concentrations at 1 to 20 second intervals depending on instrument. From 2 to 5 minutes of sample time are typically needed per chamber once the sample lines have been flushed with headspace gas, which can take as little as 1 minute.

For syringe samples, vial contents must be analyzed in the lab using a gas chromatograph connected to an electron capture detector (ECD) for N_2O analysis, a flame ionization detector (FID) for CH_4 analysis, and either a thermal conductivity detector (TCD) or an infrared gas absorption (IRGA) analyzer for CO_2 analysis. Lab-based flow-through analyzers can also be used to analyze vial contents. Autosamplers with valving that allows sequential gas analysis can efficiently analyze all three gases at once. CO_2 analysis is especially useful for detecting sampling anomalies – the absence of a linear CO_2 increase in the chamber headspace usually indicates chamber or vial leakage unless soils lack biological activity for some reason like freezing temperatures.

The choice of method will be dictated by available instrumentation and labor. In general, flow-through analyzers provide greater sensitivity but are expensive, require more expertise in the field, and for large sample campaigns can take more time in the field than syringe sampling. Sampling time in the field is dependent on the number of field personnel for the syringe method and, for the flow-through analyzer method, also dependent on the number of available analysis units. Several field personnel can sample more chambers at one time per sampling period than can one technician with a flow-through analyzer. This is an important consideration when attempting to sample multiple chambers quickly at a consistent time of day.

Chamber placement

Place chambers within plots or fields to represent existing spatial variability. In topographically uniform experimental plots, this usually means ensuring that the chambers correctly represent row–interrow areas and proportionally sample areas where fertilizer, manure, or compost are banded or injected. Depending on the size and shape of the chamber, more than one chamber may be necessary to accommodate variability arising from row-interrow and fertilizer placement. In fields, this principle also means calibrating for topographic positions and/or areas of high and low productivity. Placement is more complicated in grazed systems because sampling sites must account for animal movement (including congregation) and patches of urine and dung (including legacy effects). Chambers will periodically need to be removed to facilitate field operations. Reinstallation should be near the original sampling locations, while avoiding the exact location of prior chambers, and, where possible, waiting a day or longer to avoid sampling the disturbance associated with chamber installation.

Sample timing and frequency

Collect samples on a regular year-round basis supplemented with times of anticipated high fluxes. In cropland systems, this principle generally means weekly during the growing season with the potential for more intensive sampling at tillage, fertilization, cover crop incorporation, and substantial rainfall or irrigation following dry periods. In grazing land systems, this principle generally means more intensive sampling during active grazing, following rainfall after dry periods, or after prescribed fires. Sampling frequency can be relaxed to biweekly or even monthly during seasons with very low fluxes such as extended dry periods and deep winter. If the object of sampling is to detect long-term treatment differences rather than to construct annual budgets, then it may be possible to relax sampling frequencies somewhat.

A general regular schedule is to sample weekly during the growing season (with greater frequency following N fertilization events—see table below), every other week in early and late seasons, and monthly in winter. Sample all treatments and chambers at each sampling event to avoid biasing cumulative fluxes, which may result from integrating fluxes over different time intervals or unequal sample sizes. For annual crops, the early season is spring thaw (or another end-of-winter event) to first field operations; the growing season is first field operations to peak crop biomass; the late season is peak crop biomass to winter onset; winter is characterized by near-continuous cold. For perennial crops and pastures, the early season is spring thaw (or another end-of-winter event) to plant green-up; the growing season is green-up to peak cumulative biomass; the late season is peak cumulative biomass to the onset of winter; winter = near-continuous cold. Peak cumulative biomass accounts for forage systems that are harvested multiple times per growing season.

Ancillary measurements

Useful ancillary measurements at the time of sampling include air and soil temperature and soil moisture; it can also be useful to sample for soil inorganic nitrogen content occasionally, especially following fertilization events. Chamber and ambient air temperatures can be different with some chamber designs, so this should be checked for a given design using temperature probes on a sunny day. Chamber air temperature is a required variable for gas flux calculations. Chamber height should also be measured in three to four locations along the inside perimeter of each chamber at sampling as the insertion depth of chambers can differ and settling can occur between sampling events; headspace volume is a crucial calculation term. The preferred soil sample depth is 0-10 cm for soil temperature and moisture; for soil nitrogen 0-10 or 0-25 cm is preferred but any depth to less than the A horizon thickness is acceptable. Soil moisture and temperature should be sampled outside of the chamber but within a 1 meter distance, distance in an area without foot traffic. Soil moisture probes can be placed permanently inside the chamber footprint, with care taken to minimize soil disturbance when connecting probes for recording data.

Table 1. Summary of recommendations or measurement of chamber-based emissions of nitrous oxide (N_2O) and methane (CH_4) from cropland and pasture.

	A	B	C	D	E	F
	System	**Scale**	**Attribute**	**Minimum**	**Preferred**	**Comments**
	Cropland	Plot	Number of chambers	1-2 chambers per replicate plot placed to capture row–in		Better to sample more replicate plots than any replicat

	A	B	C	D	E	F
				terrow and fertilizer/manure bands		e plot more intensively than 1-2 chambers
			Frequency	Early season - every two weeks		Early season is from spring thaw or the equivalent end-of-winter event to the beginning of growing season
				Main growing season - weekly	Increase to twice weekly for two weeks after fertilizer or manure is applied	Growing season is from the first field operation to peak biomass
				Late season - every two weeks	Increase frequency following substantial rainfall	Late season is from peak biomass to the onset of winter
				Off season - monthly	Increase to include winter thaws or substantial rainfall	Winter is near-continuous cold
			Covariate samples	Soil moisture, air or chamber temperature	Also soil temperature, soil inorganic N	Depth: 0-10 cm for soil moisture and temp; 0-10 or 0-25 cm for soil N or another consistent depth not to exceed A horizon
	Cropland	Field	Number of chambers	As above but also sufficient to capture topographic trends		Better to sample more replicate fields than any replicate field more intensively, but topography and texture patterns will likely require chambers to be stratified at different places in any given field
			Frequency	See above		
			Covariate samples	See above		
	Grazing lands	Field	Number of chambers	Sufficient to capture the effects of topographic position and areas of h	Considerelectrical conductivity (EM) survey with directe	Better to sample more replicate pastures than any replicate pasture

	A	B	C	D	E	F
				igh vs. low pro ductivity	d sampling de sign	more intensiv ely, but topogr aphy and text ure patterns w ill likely requir e chambers to be stratified at different place s in any given pasture
			Frequency	Early season - every two wee ks		Early season s tarts with spri ng thaw or the equivalent en d-of-winter ev ent
				Main grazing season - week ly	Increase to tw ice weekly for two weeks aft er/if fertilizer or manure is a pplied	Areas where li vestock congr egate (water s ources/trees/f eeding station s) may require an increase in chamber num bers and sam pling frequenc y. Adjust frequ ency to accou nt for key eve nts associate d with grazing, haying, and/or fire managem ent
				Late season - every two wee ks	Increase to in clude substan tial rainfall	Late season is from peak bio mass to the o nset of winter
				Off season - monthly	Increase to in clude substan tial rainfall	Winter is conti nuous cold
			Covariate sam ple	Soil moisture, air or chambe r temp	Also soil tem p, soil inorgani c N	Depth: 0-10 c m for soil moi sture and tem p; 0-10 or 0-25 cm for soil N or another de pth not to exc eed A horizon

Materials

Gas sampling:

- Static chamber bases of an appropriate size; Kahmark et al. (2020) provide plans for a 29 cm diameter stainless steel cylinder with clips to fasten an air-tight lid; see Parkin and Venterea (2010) for additional designs, including a rectangular chamber with water-filled channels to seal lids.
- Chamber lids with an O-ring or other air-tight seal to fit chamber, drilled with a hole to accept a butyl rubber septum for syringe sampling and / or fitted with two bulkhead unions for attaching headspace circulation tubing.

- For syringe sampling:

1. Septa for chamber lids scavenged from 10 mL Vacutainer serum vials (e.g., Becton Dickson #366430); septa should be replaced frequently.

Equipment	
BD Vacutainer	NAME
Serum Tubes	TYPE
BD	BRAND
366430	SKU
https://www.bd.com/en-us/products-and-solutions/products/product-page.366430	LINK

2. Airtight sample vials, e.g., Exetainer, 5.9 mL flat bottom with septum cap and septa, available from Labco, **https://www.labco.co.uk/**

3. Plastic syringe with Luer-lok tip, 10 mL (e.g., Becton Dickinson, #309604)

Equipment	
SYRINGE, LL 10CC (100/BX)	NAME
SYRINGE	TYPE
BD Luer-Lok™	BRAND
309604	SKU
https://mms.mckesson.com/product/127230/BD-309604	LINK

4. Hypodermic syringe needles, 1” 22 gauge (e.g. Becton Dickinson #305155)

Equipment	
BD™ Needle 1 in. single use, sterile, 22 G	NAME
Needle	TYPE
BD	BRAND
305155	SKU
https://www.bd.com/en-ca/products-and-solutions/products/product-page.305155	LINK

5. Stopwatch

6. A means to record for each chamber the interior height at several locations as well as air temperature, soil temperature, sample times, soil moisture

In situ gas analysis for flow-through analysis:

1. Portable N_2O and CH_4 analyzers, e.g. Licor LI-7820 N_2O/H_2O Trace Gas Analyzer and Licor LI-7810 $CH_4/CO_2/H_2O$ Trace Gas Analyzer, LI-COR, Inc. Lincoln, NE.
2. A means to record for each chamber the interior height at several locations, air temperature, soil temperature, sample times, soil moisture.

- Soil moisture probe, e.g. Hydrosense (Campbell Scientific, Logan UT) or a soil push probe to collect soil samples for gravimetric moisture.
- Soil push probe to collect samples for soil inorganic N analyses (e.g., Oakfield Model LS (Oakfield Apparatus, Fond du Lac Wisconsin) or JMC Model PN031 (JMC Soil Samplers, Newton IA) or equivalent.
- Plastic bags for soil samples.
- Insulated cooler with ice packs to transport soil samples for inorganic N analyses to laboratory.

Laboratory gas analysis for syringe samples:

- Gas chromatograph equipped with a ^{63}Ni ECD for N_2O, FID for CH_4, IRGA or TCD for CO_2, and autosampler (e.g. Agilent Model 7890A coupled to a Gerstel MPS2XL autosampler); alternatively, lab-based flow-through analyzers as noted above can be used for gas analyses by fitting flow paths with injection ports.

- Four to seven duplicate analytical standards (N_2O, CH_4, CO_2) at concentrations that bracket expected concentrations in vials; typically these are 300 to 900 ppb_v for N_2O, 1 to 5 ppm_v for CH_4, and 400 to 1200 ppm_v for CO_2. Check standards should be run often (e.g., every 20 samples) to track potential instrument drift, to examine differences between two columns on a dual column ECD setup, and to locate and solve instrument issues. Coefficient of variation (CV) can be calculated from the check standards.

Sample Collection

20m 30s

1 **Chamber deployment (minimum of one day before sampling; one week preferred):**

Place chamber bases in representative positions and pound into the soil ~5 cm using a flat board and mallet or other technique. Disturb the soil and surrounding plants as little as possible. As needed, clip plants to below top of chamber. A detailed working protocol is available in Kahmark et al. (2020).

1.1 **Prepare lids for gas sampling – for syringe sampling, replace the rubber septum as needed:**

- For portable analyzer sampling, calibrate/span occasionally and check for accuracy against typical ambient concentrations before using.
- Make sure tubing is not crimped or blocked and sample ports on lids are clear.

2 **Gas sampling:**

- Gas sampling should occur at about the same time of day on each sample date, and the sampling sequence should be staggered by treatment (i.e. do not sample all replicates of one treatment together) in order to avoid any systemic time-of-day bias.
- Be careful to minimize trampling around chambers.
- A detailed working protocol with helpful visuals is available in Kahmark et al. (2020).

2.1 **Measure and record:**

- Chamber height at 3-4 locations around its perimeter; these measurements are used to determine the chamber volume, important for areal flux estimates.
- Soil and air temperature adjacent to chamber or, if chamber air temperature is different from ambient, air temperature within chamber at beginning and end of the closure period.
- Soil moisture adjacent to chamber.
- Time of day and time at which individual chambers are sampled if different than predetermined interval.

2.2 **For syringe sampling:**

- Install the lid
- Add a vent needle to the first vial
- Insert the sampling syringe needle into the chamber lid septum and mix the chamber headspace by gently pumping the syringe three times; then remove at least 10 mL of headspace gas.
- Inject the headspace sample into the vented sample vial to flush the vial with sample. Repeat for a total of three flushes. After flushing, remove the vent needle.
- Re-insert the sampling syringe needle into the lid septum, pump the syringe three times to mix the chamber headspace, then withdraw at least 10 mL, inject into the sample vial, and

record the stopwatch time. Adding 10 mL to a sample vial already at atmospheric pressure will over-pressurize the vial in order to prevent sample contamination, allow for the detection of vial leaks prior to analysis, and may be needed to flush the injection port of GCs with attached autosamplers. Samples should not be stored in plastic syringes because syringes can absorb and emit methane.

- Repeat chamber sampling an additional three times at ~15 minute intervals (or other pre-determined interval time), recording the stopwatch time each time a headspace sample is removed from the chamber. Between intervals several other chambers can be sampled.

2.3 **For portable analyzer sampling:**

- Most analyzers require a warmup time. Consult the manufacturer's manual for recommended time frames and pre-test.
- Connect the lid and tubing assembly to the analyzer input and output sampling ports .
- Attach the lid to the chamber base and allow the system to recirculate with sufficient time to ensure chamber sample is entering the analyzer (~30 seconds depending on tubing length and flow rate). Sample for as long as needed to record a stable flux. Typically, the rate of change in concentration starts to stabilize in about a minute, after which ~2 minutes of additional sampling is required. If fluxes fail to stabilize after 3 minutes, suspect poor chamber closure or fluxes below detection limits.

Flux Calculation

3 **For syringe samples:**

- Calculate a volume-based flux (α_v) from the linear relationship between headspace gas concentration (ppm_v or µL gas/L headspace) and time of sampling (min), to yield a flux of µL gas/L headspace/min.
- In these calculations gas is either N_2O-N, CH_4-C, or CO_2-C. If fluxes are non-linear a non-linear flux calculation method may be necessary; see Venterea et al. (2020).

3.1 Convert α_v with units based on volume to α_m with units based on mass, in microgram per liter per minute, and correct for field temperature using the following application of the Ideal Gas Law:

$$\alpha_m = (\alpha_v \times M \times P) / (R \times T)$$

where:
α_m is expressed in µg N or C/L/min
M = molecular weight of GHG (28 µg N/µmol N_2O or 12 µg C/µmol CO_2 or 12 µg C/µmol CH_4)
P = assumed atmospheric pressure = 1 atm
R = Universal gas constant = 0.0821 L-atm/mol-K = 0.0821 µL-atm/µmol-K
T = field temperature, in °K = °C + 273

4 **For portable analyzer samples:**

Determine time from chamber closure to even mixing of headspace (deadband) and remove from flux calculation.

4.1 Determine the units reported by the analyzer and convert to α_m: ug N_2O-N/L/min for N_2O, ug CH_4-C/L/min for CH_4, and ug CO_2-C/L/min for CO_2.

5 **For both syringe samples and portable analyzers:**

From α_m, calculate the gas flux (f_m) as microgram of element (N for N_2O; C for CO_2 and CH_4) per square meter per hour), using the equation:

$$f_m = (\alpha_m \times V \times 60 \text{ min/h}) / A$$

where:

f_m is expressed in µg N or C/m^2/h

α_m = as above, in µg/L/min

V = volume of gas in chamber, in L

A = soil surface area covered by chamber, in m^2

5.1 **Report:**

- for N_2O: µg N_2O-N m^{-2} h^{-1}
- for CH_4: µg CH_4-C m^{-2} h^{-1}
- for CO_2: mg CO_2-C m^{-2} h^{-1} ; note that CO_2 is in mg not µg – typically fluxes of CO_2 are much higher than fluxes of N_2O and CH_4; convert to mg by multiplying f_m by 1000.

Upscale values as needed or appropriate, e.g., to report values as g ha^{-1} d^{-1}, multiply µg m^{-2} h^{-1} by 0.24.

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