References for Field Measurement of Greenhouse Gas Emissions


USDA-ARS GRACEnet Project Protocols  
Chapter 3. Chamber-Based Trace Gas Flux Measurements  
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Scope:  
This protocol addresses N2O, CO2 and CH4 flux measurement by soil chamber methodology. The reactivities of other gasses of interest such as NOx, O3, CO, and NH3 will require different chambers and associated instrumentation. Carbon dioxide is included as an analyte with this protocol; however, when plants are present, interpretation of soil CO2 flux data in the context of net GHG flux is not straightforward because soil CO2 emissions do not represent net ecosystem CO2-C exchange. This protocol adopts chamber-based flux methodology (the least expensive option available) in order to allow inclusion of as many sites as possible. Since micrometeorological techniques require expensive instrumentation, they will be used only at locations with current micrometeorological capability. In deciding on a chamber design, our goal was to adopt methodology which is sensitive, unbiased, has low associated variance, and allows accurate interpolation/extrapolation over time and space. Because of our inability, at this time, to precisely assess the extent of bias associated with a given chamber design and sampling protocol under the range of conditions which might exist, we have adopted our 'best guess' protocol. Assessment, refinement and/or modifications of this protocol may continue in the future. At some sites this may include evaluation of chambers against fluxes determined by micrometeorology or performing comparisons of alternate chamber designs. Recognizing that any measurement technique will have disadvantages, the best we can do at this time is to select a technique which minimizes potential problems. In addition, adoption of common methodology will aid in site inter-comparisons. To facilitate the adoption of a common technique, it is important to attain a common understanding of the potential shortcomings associated with chamber-based flux measurement techniques (Rochette and Eriksen-Haamel, 2008). The following section summarizes some of these issues.

Considerations for Chamber Construction and Deployment.  
Several issues related to chamber techniques for gas flux measurement must be considered. These are discussed below along with recommendations to minimize potential problems.

1. Soil Disturbance: In the short term soil disturbance can occur upon installation of the anchor used to support the chamber. Longer term microclimate effects within the anchor have also been observed. Installed anchors may retain water and become flooded during high precipitation.

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events. Humidity within the anchor may facilitate algal growth on the soil surface. Shading by the anchor may alter the temperature regime of the soil.  

**Recommendations:** Install permanent chamber anchors at least 24 h prior to flux determinations. Minimize anchor or collar height to reduce micro environment perturbations. Move chamber anchors if soil microclimate effects are observed. Venterea et al. (2010) describe a chamber design using anchors that are nearly flush with the soil surface and thus would minimize microclimate effects (See Appendices III and IV). Pump excess or flooded water from chamber anchors as soon as possible. In situations where it is not feasible for chamber anchors to be installed in the soil, use temporary/portable chambers with a wind skirt (Matthias et al., 1980) may be considered.

2. Temperature perturbations: Temperature differences can have a marked effect on biological activity, and also cause gas expansion/contraction which can complicate flux calculations and create experimental artifacts if appropriate temperature corrections are not made. Absorption or dissolution of dissolved soil gasses is also impacted by temperature. The goal is to have the temperature regime within the chamber as similar as possible to the external temperature.  

**Recommendations:** Use insulated chambers to help maintain a constant temperature regime during deployment. Reflective material can be used to construct or coat the chamber to reduce absorption of sunlight (e.g. double reflective insulation by Reflectix, Inc., www.reflectix.com). Keep chamber deployment time as short as possible without sacrificing detection sensitivity. Install thermocouple or thermometer in the chamber lid to monitor temperature changes throughout the incubation period.

3. Pressure perturbations: Wind passing over the chamber anchor may cause pressure-induced mass flow of gas into or out of the soil. Closed chambers placed on the soil can reduce natural pressure fluctuations. Sampling of gas within the chamber can cause mass flow of gas from the soil.  

**Recommendations:** Pressure and sampling perturbations can be reduced using a properly vented closed chamber (Hutchinson and Mosier, 1981; Hutchinson and Livingston, 2001; Xu et al., 2006).

4. Humidity perturbations. Humidity increases due to deployment of a chamber on the soil will effect trace gas concentrations in the chamber due to dilution by water vapor. For example, at 25°C and 100% relative humidity, the maximum concentration of water vapor in the air is 0.0305 L/L. Thus, if the ambient relative humidity was zero, and placement of a chamber resulted in an increase in relative humidity within the chamber from 0% to 100%, the maximum dilution of other gases would be 0.0305 L/L or a dilution of 3.05%. The potential underestimation of trace gas concentration in the chamber headspace due to dilution does not necessarily mean that the flux will be underestimated. If dilution changes the degree of curvi-linearity of the time course data, gas flux could be overestimated. The magnitude (and direction) of this effect depends on the magnitude of the soil gas flux as well as the chamber headspace height. In addition to trace gas dilution by water vapor other potential humidity effects include changes in soil water which could impact both soil biological activity and the amount of gas dissolved in the aqueous phase. Water vapor can also cause interferences in detection of other gases if a photoacoustic or other infra-red based analyzer is used.
**Recommendations:** Keep chamber deployment short. Relative humidity changes inside chamber could be estimated and used to potentially correct for dilution and/or gas solubility effects.

5. Gas Mixing: It is generally assumed that molecular diffusion is sufficiently rapid within the chamber headspace such that homogeneous gas concentrations exist when sampling (Livingston et al., 2006). However, this may not necessarily be true if large amounts of vegetation are present or the chamber volume to surface area ratio is large.

**Recommendations:** If it is deemed that mixing of the headspace gas is necessary, the best option is to fit the inside of the chamber with a gas distribution manifold connected to the sampling port. The manifold has a single port on one end (which extends out the top of the chamber) and multiple ports on the other end which accept small diameter teflon tubing (e.g., 1/16") that extend into the chamber. The narrow tubing from each of the multiple inner ports is extended to different points inside the chamber, so that when the sample is collected, gas is pulled from multiple points in the chamber. Manifolds can be purchased from Small Parts, Inc. (www.smallparts.com). An example part no. is B000P7KZ9Y (description = Stainless steel hypodermic tubing manifold, inlet - 13 Gauge, 6 outlets - 20 Gauge). The recommendation of placement of a small fan within the chamber, made in the previous version of this protocol is not advised. It has been observed that fans can induce pressure perturbations within the chamber.

6. Chamber Placement: In row-crop systems it is important that chambers be deployed to adequately represent the system. For smaller chambers this will necessitate placement of chambers in both the row and inter-row areas of the plot. Alternatively, chambers with a larger footprint that provide more representative coverage of the system under study can be used, ideally utilizing chambers designed to cover the entire inter-row area. One goal of the GRACEnet project is to quantify ecosystem contributions to net trace gas flux; therefore, plants should be included inside chambers during flux determinations. There is some information indicating that N\textsubscript{2}O emission may be facilitated by living plants (Chang et al., 1998; Chen et al., 1999; Smart and Bloom, 2001), however, this effect has only been observed under flooded conditions. Chambers must also be placed to sample other representative features of the system under study (e.g. tillage or fertilizer bands).

**Recommendations:** Inclusion of plants presents several problems. With regard to sensitivity, inclusion of plants would likely dictate that chamber height be increased, but an increase in chamber height results in an increase in chamber headspace volume and a corresponding decrease in flux detection sensitivity (minimum detectable flux limit is described below). Significant reductions in sensitivity might, in some cases, result in all the flux measurements being below the detection limit. In such cases, it is advisable to also measure bare soil fluxes (i.e. between rows in row-crop agriculture) using shorter chambers which have higher sensitivity. Results could then be reported as fluxes within a range of the bounds established by the two measurements. If it is not feasible to include plants at all growth stages, at least deploy chambers both within and between rows (in row crop agriculture). Inclusion of plants complicates interpretation of CO\textsubscript{2} flux data. Production of CO\textsubscript{2} by living plant tissue (both above and below ground) contained within the chamber cannot be considered in estimates of total GHG production unless annual photosynthetic CO\textsubscript{2} uptake is also measured. Finally, when small chambers are used to sample distinct areas of the field that are not, in and of themselves, representative of the entire field (e.g. soil containing a fertilizer band vs. non-fertilizer band soil),
then a mathematical weighting of the fluxes must be performed in order to obtain the average field or plot flux.

7. Frequency and timing of flux measurements. Trace gas fluxes exhibit a high degree of temporal variability. Thus, the more frequently measurements are made, the more accurate the integrated seasonal/yearly cumulative flux estimate will be (Smith and Doobie, 2001; Parkin 2008). There are several components of temporal variability that must be considered including: i) diurnal variations, ii) seasonal variations, and iii) variations induced by perturbation (e.g., tillage, fertility, irrigation/rainfall, thawing).

**Recommendations:** To account for diurnal variability, measure flux at times of the day that more closely correspond to the daily average temperature (mid morning, early evening). A Q$_{10}$ temperature correction procedure may be used to adjust rates to the average daily temperature, but caution is warranted. The temperature correction procedure assumes that temperature variations are the primary factor driving diurnal flux variations, an assumption that may not be universally true. Selection of both the appropriate Q$_{10}$ factor and the soil temperature (depth) to be used are critical. The time lag between gas production in the soil profile and gas flux from the soil surface will dictate the appropriate soil temperature to use in performing the Q$_{10}$ flux correction. Finally, a wide range of Q$_{10}$ values for N$_2$O have been reported in the literature (Brumme et al., 1999; Dobbie et al., 1999; Dobbie and Smith, 2001; Machefer et al., 2002), so critical determination of the appropriate Q$_{10}$ factor must be done. It is recommended that if a Q$_{10}$ correction is performed, the original non-corrected fluxes should be reported as well. To account for perturbation-induced variations is recommended that fluxes be measured as soon as possible after the perturbation (such as rainfall, tillage, or fertility event), then daily for the next several days during and following the specific event. During the remainder of the year, gas flux measurements should be made at regular time intervals (every 1 or 2 weeks). It is highly recommended that fluxes be measured at least weekly and more frequently if resources allow (Parkin 2008).

7. Spatial Variability: Trace gas fluxes exhibit a high degree of spatial variability, and Coefficients of Variation associated with chamber-based fluxes commonly exceed 100%. Variability may also be a function of chamber size, and may be reduced by using larger chambers. Use of larger chambers can result in the physical ‘averaging’ of microsites, thus reducing variability (Parkin, 1987; Parkin et al., 1987). **Recommendations:** Use chambers with larger footprint to minimize small scale variability. Use as many chambers as possible. It is recommended that a minimum of two chambers per plot in plot scale studies. In landscape or field studies it is recommended that ‘similar’ landscape elements be identified and a sampling design employed where chambers are stratified by landscape element, soil type, or vegetation (Livingston and Hutchinson, 1995). In situations where identifiable hotspots may occur (e.g., urine patches in a grazed system) a sampling design will have to be developed to account for this. Gilbert (1987) gives some sampling guidelines when hotspots exist.

**Recommended Protocol**
Gas flux will be measured by static chambers deployed on the soil surface for a period of typically no more than 60 min. During chamber deployment, samples of the chamber headspace gas will be removed at regular intervals, and stored for later analysis by gas chromatography. Specific recommendations on chamber design, gas sampling and analysis, and flux calculations
are provided below. Investigators are encouraged to examine the referenced literature underlying these recommendations.

**Minimum Requirements for Chamber Design:**
1. Flux chambers should be fabricated of non-reactive materials (stainless steel, aluminum, PVC, polypropylene, polyethylene, or plexiglass.)
2. Material should be white or coated with reflective material, (mylar or painted).
3. Chambers should be large enough to cover at least 182 cm² of the soil surface, and have a target height of 15 cm (height can be decreased to increase sensitivity or increased to accommodate plants).
4. Chambers should contain a vent tube, at least 10 cm long and 4.8 mm in diameter (e.g., 1/4” stainless steel tubing). See Fig. 1 for details. Alternatively, Xu et al. (2006) describe a novel circular vent tube designed to completely eliminate wind-induced pressure gradients.
5. Chambers should have a sampling port to enable the removal of gas samples. Possible options include: butyl rubber septa or a nylon/polyethylene stopcock.

**Recommended Design:**
Chambers should have two parts; a permanent anchor, driven into the soil and a flux chamber cap which contains the vent tube and sampling port. Anchors are fabricated so that they can accommodate the flux chamber during measurement phase. Anchors and chambers can be made of 20 cm (or larger) diameter PVC. Alternatively, anchors can be made of thin-walled stainless steel or aluminum to minimize physical disturbance upon insertion. The vent tube is necessary to avoid pressure perturbations (and subsequent mass flow) when chambers are installed on the anchor, and when gas samples are collected. Photographs of several chamber designs are presented in Appendix III and descriptions of chamber construction are provided in Appendix IV. Some of the supplies and vendors of materials for chamber construction are provided in Appendix II.

**Chamber deployment**
**Anchors:** Anchors should be installed at least 8 cm into the ground and extend no more than 5 cm above the surface. Permanent anchors should be installed at least 24 h prior to first flux measurement. There are no fixed guidelines regarding how long anchors can (or should) be left in place. In cultivated systems, chamber anchors are typically removed prior to cultivation, planting, or fertilizer application, and then replaced. In grassland studies anchors have been left for over 10 years with no apparent deleterious effects. One advantage of leaving anchors in
place is that soil disturbance and root damage are minimized. However, there have been reported problems with microclimate effects within the anchors left in place for extended periods. For example, changes in humidity or shading can cause algal growth, and in heavy or compacted soils ponding of rainwater can occur. This is not a desirable situation. It will be up to the investigator to determine how often chambers should be moved.

**Gas sampling:** Fluxes are measured by determining the rate of change of trace gas concentration in the chamber headspace. In most cases trace gas concentrations are determined by physically removing a gas sample from the chamber headspace for analysis in the laboratory. Gas samples should be withdrawn at regular intervals during the chamber deployment. Chambers should be in place no longer than 60 minutes. The shorter time the deployment time, the smaller the chamber-induced biases, but deployment must be long enough so that sensitivity is not compromised. At least 3 time points are required for flux calculation: time 0, and two additional points, equally spaced in time (e.g. 0, 30, 60 min. or 0, 20, 40 min). [Note: Sampling is performed at regular intervals to facilitate flux calculation by Eq. 1. However, more samples can be collected, and models exist for the analysis of data not collected at equi-spaced time intervals. see Flux Calculation Section, below. Using more than 3 time points will decrease uncertainty in flux calculations, but with an obvious trade-off in additional labor]. Sampling is performed by inserting a polypropylene syringe into the chamber septa and slowly removing a gas sample. Mixing of headspace gas by pumping the syringe before sampling is not recommended as pumping may cause pressure perturbations and/or excess dilution of headspace gas by entry of outside air through the vent tube. The gas volume removed at each time point is dictated by the specific gas analysis technique to be used. Typically, from 5 to 30 ml are removed. If the syringe is equipped with a stopcock, the sample can be stored directly in the syringe for a short time. Alternatively, the gas sample can be transferred to a previously evacuated glass vial sealed with a butyl rubber septum. It is recommended that enough gas be injected into the evacuated vial to produce an overpressure. This overpressure facilitates the subsequent removal of a gas sample for analysis. It should be noted that each time a headspace gas sample is removed from the chamber outside, air flows into the chamber through the vent tube. This results in a dilution of the analyte in the chamber headspace. The error
associated with this dilution effect is a function of both the sample volume withdrawn and the chamber Volume/Surface Area ratio (Figure 2). Correction for this dilution effect should not be necessary for chamber Volume/Surface Area ratios >10 and sample volumes < 30 ml. Prolonged storage (>2 d) of gas samples in polypropylene syringes is not recommended as leakage can occur. An example of a gas sampling protocol is presented in Appendix I. In some situations instrumentation may be available for real-time analysis of headspace gasses. Infra-red gas analyzers have been successfully used for determining soil CO₂ fluxes. Photoacoustic analyzers reportedly can measure a suite of gasses (e.g. CO₂, N₂O, CH₄, H₂O). However, we recommend extreme care in such circumstances. The combination of the overlap of the absorption spectra of the different analytes, combined with the large range in analyte concentrations (e.g. CO₂ ~ 380 ppm vs. N₂O~ 320 ppb) in air pose potential problems for precise estimation of fluxes. Interferences of water vapor with CH₄ determinations have been noted (Parkin, unpublished). Similarly, CO₂ interferences with N₂O have been observed (Akdeniz et al., 2009). If photoacoustic analyzers are used it is highly recommended that calibrations be performed with mixed gas standards where the relative concentrations of the different gasses are changed. Alternatively, if N₂O is the analyte of interest, a soda lime trap can be installed to scrub CO₂ from the gas sample stream.

Vials, septa and storage: Brooks (1993) evaluated several storage protocols and found that red rubber stoppers such as found on commercially available evacuated blood vials were the worst. Parkin (1985) observed that red rubber absorbed N₂O. Recently, Glatzel and Well (2008) tested the integrity of red rubber stoppers, grey butyl rubber stoppers, and Exetainer vials (with grey butyl rubber septa) after repeated needle punctures. These investigators observed pressure losses of approximately 94%, 84% and 30% following 5 needle punctures of butyl rubber stoppers, red rubber stoppers, and Exetainer septa, respectively. However, we have observed marked differences in quality of grey butyl rubber stoppers obtained from different vendors. We repeated the Glatzel and Well experiment with two batches of grey butyl rubber stoppers on glass crimp-top serum vials and with Exetainer vials. The septa or stoppers were punctured 5 times with a 22 gauge needle and the vials were then injected with room air to achieve a 250 mBar overpressure. Pressure in the vials was determined after 3 d and 13 d (at room temperature and pressure) with a pressure transducer. In our experiment the Exetainer vials (Labco Limited part # 938W) and 6 ml serum vials (Alltech Associates) with grey butyl rubber septa obtained from Voigt Global (part # 73828A-RB) maintained > 90% of the overpressure for 13 d, and had low variability (Fig. 3). However, average pressure retention of the Grace stoppers was 71% at 3 d, and dropped to 60.8% at 13 d. The Grace stoppers were also highly variable, as indicated by the standard deviation bars. Rochette and Eriksen-Haamel (2008) characterize the use of Exetainer vials (which have grey butyl rubber septa) as the “best” practice, and use of vials with other butyl rubber stoppers as ‘good’. We agree with this recommendation. In our evaluation of butyl rubber stoppers obtained from a variety of different vendors we observed marked differences in efficacy of sample integrity for different batches of butyl rubber stoppers. Because of the variability in the quality of butyl rubber stoppers on the market, it is highly recommended that the rubber stoppers of each new batch be tested for the ability to hold vacuum and pressure. Additionally, we have observed that gas leaks can occur around the stopper in crimp top serum vials if the crimp is not applied tightly. When a manual crimping tool is used variability among individuals can be a factor and care must be taken to assure adequate crimping pressure. A recommended strategy for dealing with gradual loss of pressure
from vials requiring storage prior to analysis is to overpressurize them initially (as recommended above), and then immediately prior to analysis de-pressurize them by inserting a small needle through each septum for a few seconds. This procedure will ensure that all vials are at the same (ambient) pressure at the time of analysis, and also allows for an assessment of sample integrity (i.e., samples that have not held pressure are suspect). This procedure also accounts for the fact that different vials are likely to leak at different rates. Standards must be treated in the same way, and the sample injection system (e.g., autosampler) used must be capable of handling samples at ambient pressure. This procedure has been used successfully over several years (Venterea et al., 2005; 2010).

Gas Analysis: Samples should be run as soon as possible after collection. Gas chromatography will be used for analysis of N₂O and CH₄ (electron capture detector for N₂O and flame ionization detector for CH₄). Specific method of gas sample injection into the GC will depend upon the specific instrumentation available at each location. However, it is recommended that the GC be fit with a sample valve to minimize injection error and thus increase analytical precision. To account for problems associated with GC drift it is recommended that: i) samples from individual chambers are run in sequence (e.g. t₀, t₁, t₂,) rather than segregating all the samples by time (i.e. all t₀ samples run in sequence, then all t₁ samples run in sequence, etc.) and ii) standards are run periodically throughout the sample run (e.g. every 10 to 20 samples).

Standards: Standards should be prepared each sampling time. Standards should be handled in a manner similar to samples with regard to collection and storage. Preferably samples should be prepared in the field (i.e. injected into glass vials, or collected in syringes). Several different standard concentrations should be run, as detector response may be nonlinear. The range of standards should bracket the concentrations found in samples [e.g., N₂O; 0.1, 1.0 and 10 ppm; CH₄; 0.5, 1, 2, and 10 ppm]. Standard curves are then used to convert the GC output of the samples into units of ppm. It has been noted that occasionally the stated concentration on purchased standard gasses may be erroneous (A. Mosier, pers. comm.). With some gas chromatographs, oxygen has been observed to influence N₂O detection sensitivity when measured with an electron capture detector (Parkin, unpublished). The specific gas chromatograph used should be checked for this effect, and the make-up gas of all standards should reflect the gas composition of the atmosphere (i.e. approx. 20% O₂ and 78% N₂). It is recommended that funds be allocated in the GRACEnet project to purchase a common NIST-certified standard gas mixture to be used to check standards at all locations.

Data Analysis
Flux Calculations: Fluxes are calculated from the rate of change of the concentration of the analyte of interest in the chamber headspace. Since the units associated with the gas standards will typically be ppm, when the standard curve relationship is applied to calculate gas concentrations of the samples, the resulting unit of the analyte is also ppm. The units, ppm, are typically on a volume per volume basis (which is the same as a mole per mole basis). Volumetric parts per million (ppm(v)) has units of μL trace gas L⁻¹ total gas. For example a 1 ppm (vol/vol or mol/mol) N₂O standard will contain 1μL N₂O / L of gas. If the rate of change of headspace trace gas concentration is constant (i.e. ppm(v) vs. time data is linear), then linear regression can be used to calculate the slope of the concentration vs. time data. The slope of the line is the trace gas flux. Thus, a regression of ppm(v) vs. hours will result in a slope with units of μL gas L⁻¹ h⁻¹.
Multiplying the slope by the chamber volume (L) and dividing by the chamber surface area (m²) will result in a flux with units of \( \mu L \) trace gas m⁻² min⁻¹. If the rate of change of headspace trace gas concentration is not constant (i.e. ppm(v) vs. time data is curvi-linear), then linear regression may not be appropriate. Curvi-linear concentration data with time is attributed to a build up of the analyte concentration in the chamber headspace (Hutchinson and Mosier, 1981), which alters the diffusion gradient and the resulting flux (Hutchinson and Mosier, 1981), or to horizontal movement of gas in the soil (Livingston and Hutchinson, 1995), or to leakage from the chamber (Stolk et al., 2009). To account for this effect, Hutchinson and Mosier (1981) proposed an algorithm as an alternative to linear regression (Eq. 1).

\[
fo = \frac{(C_1 - C_0)^2}{t_1 \times (2 \times C_1 - C_2 - C_0) \times \ln[(C_1 - C_0)/(C_2 - C_1)]}
\]

where \( fo \) is the flux at time 0, \( C_0, C_1, \) and \( C_2 \) are the chamber headspace gas concentrations (ppm(v)) at time 0, 1, and 2, respectively, and \( t_1 \) is the interval between gas sampling points (h). The resulting units of \( fo \) are: \( \mu L \) trace gas Liter⁻¹ h⁻¹. In order to convert these units to \( \mu L \) trace gas m⁻² h⁻¹, \( fo \) must be multiplied by the chamber volume (Liters) and divided by the chamber surface area (m²).

In addition to the Hutchinson and Mosier (HM) method, there have been several alternative methods proposed for the analysis of curvi-linear data. The quadratic procedure described by Wagner et al., (1997) involves fitting a quadratic equation to the concentration vs. time data (Quad method). The flux is then computed as the first derivative of the quadratic equation at time zero. Pedersen et al. (2001) developed a stochastic diffusion model that is an extension of the HM method and does not require equi-spaced data points, and can accommodate more than three data points. The non-steady-state diffusive flux estimator (NDFE) developed by Livingston et al. (2006) is a 3 parameter model in which \( fo \) can be derived from concentration vs. time data by non-linear regression. Recently, Pedersen et al., (2010) developed a technique designated as the HMR model, which is a modification of the Hutchinson/Mosier technique to account for horizontal gas diffusion and/or chamber leaks. Similar to the Pedersen stochastic model, the Quad method and the NDFE model, the HMR technique can be used with data sets of 3 or more points. However, as alluded to by Pedersen et al. (2010), the practice of evaluating a 3 parameter model (i.e. HMR or Quad or NDFE) with only 3 or 4 data points is not optimal. Such situations may result in parameter estimates being non-significant.

**What is the best method?** Several criteria must be considered in the selection of an analysis technique to apply to a given data set. Past studies have evaluated some of the aforementioned methods with regard to the bias (accuracy) associated with the calculated flux estimate (Livingston et al., 2006; Venterea et al 2009; Venterea 2010; Pedersen et al., 2010). However, in addition to bias, the variance associated with the calculation method must also be considered. Every analytical technique for gas measurement has an associated error. In the case of gas chromatography, the precision (coefficient of variation) of the gas measurements is often in the range of 1 to 6% when small (0.2 to 1.0 ml) gas samples are used. The error associated with gas measurement (as well as other sampling errors) can result in the occurrence of “noisy data” (Anthony et al., 1995), and this “noise” induced by sampling and analytical variability can introduce a variance component to the flux estimation method. Thus, in addition to bias, the variance of the flux estimation method should also be considered.
A statistical analysis by Venterea et al. (2009) demonstrated that clear trade-offs exist between bias and variance in selecting a flux-calculation scheme, with linear regression having greater bias but less variance compared with the HM and Quad methods. Parkin and Venterea (manuscript in preparation) investigated these issues further, using Monte Carlo simulation to evaluate the bias and variance of linear regression, the HM method, and the Quad method when applied to data sets of 3 or 4 points, with chamber deployment times of 0.5 h, 0.75 h and 1.0 hour, and different degrees of data curvi-linearity. Monte Carlo simulations were performed by constructing simulated N$_2$O chamber data using the method described by Venterea et al. (2009). This analysis was applied over a range of analytical precisions (1% to 6%). When an estimation method has both bias and a variance component, the appropriate selection criterion is the Mean Square Error (MSE) which combines the bias and variance (Eq. 2) (DeGroot, 1986).

$$\text{MSE} = \text{Variance} + \text{Bias}^2$$  \hspace{1cm} \text{Eq. 2}

Our analyses showed that there is not a simple answer to the question, “Which flux calculation method is the best?” The MSE of a given flux calculation method is dependent upon three factors: 1) the magnitude of the underlying flux, 2) the degree of data curvi-linearity, and 3) the analytical precision. For example, Fig. 4 shows the MSE associated with linear regression, the Quad method and the Hutchinson/Mosier method across a range of simulated N$_2$O fluxes for a given chamber height, deployment time, and GC precision. The points where the curves intersect indicate decision points for the different calculation methods. Below 22 $\mu$g N m$^{-2}$ h$^{-1}$ linear regression has a lower MSE than either the Quad or HM methods, thus it is the method of choice. At fluxes between 22 and 52 $\mu$g N m$^{-2}$ h$^{-1}$ the Quad method has the lowest MSE, and for fluxes > 52 $\mu$g N m$^{-2}$ h$^{-1}$ the HM method has the lowest MSE and should be used. These flux decision points are only valid for data sets with a certain degree of curvi-linearity (controlled, in part, by chamber height, deployment time, and soil characteristics), and an analytical precision of 2%. As data curvi-linearity and analytical precision change, the decision points for the different methods also change. While quantifying analytical precision is relatively straightforward, characterizing the degree of data curvi-linearity is not. Here we propose a calculation that can be

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4.png}
\caption{Mean Square Errors associated with different calculation methods over a range of simulated N$_2$O fluxes. 3-point curvi-linear data were generated with the algorithm of Venterea 2009 using a Tao value of 1.0).}
\end{figure}
used to empirically quantify data curvi-linearity as a tool to aid in selection of the appropriate calculation method (Eq. 3).

\[
\text{Data Curvi-linearity Index} = \frac{(C_1-C_0)}{(C_2-C_1)} \quad \text{Eq. 3}
\]

where \(C_0\), \(C_1\), and \(C_2\) are headspace gas concentrations for 3 equi-spaced time points (time 0, time 1, time 2). [Note: the constraint of equi-spaced time points is required for Eq. 3, however a more generalized form of the data curvi-linearity index can be calculated as the slope of the \(\frac{1}{2}\) of the time course data divided by the slope of the second \(\frac{1}{2}\) of the time course data.]

Figures 5 and 6 show the decision curves for the HM method vs. linear regression as a function of the Data Curvi-linearity Index (DCI) and the apparent \(\mathrm{N}_2\mathrm{O}\) flux. The blue curves in the figures delineate the points where the MSE of the Hutchinson/Mosier method equals the MSE of linear regression. Above and to the right of the curves, the MSE of the HM method is less than the MSE of linear regression, while in the regions below and left of the curves MSE of linear regression is less than the MSE of the HM method. In these plots, the apparent \(\mathrm{N}_2\mathrm{O}\) flux was calculated as: \((C_{\text{end}}-C_0)/T_d\), where \(C_0\) and \(C_{\text{end}}\) are headspace gas concentrations (\(\mu\text{L} \ \mathrm{N}_2\mathrm{O} \ \text{L}^{-1}\)) at time 0 and the end timepoint, respectively, and \(T_d\) is the total chamber deployment time (h). To illustrate how Figs. 5 and 6 can be used to determine which calculation method should be used for a given data set, two examples are provided. In example data set 1, the DCI is calculated to be 1.224, and the apparent flux is calculated to

**Example Data Set 1**: \(\mathrm{N}_2\mathrm{O}\) time course chamber data.

<table>
<thead>
<tr>
<th>Headspace</th>
<th>(\mu\text{L} \ \mathrm{N}_2\mathrm{O} \ \text{L}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.319</td>
</tr>
<tr>
<td>0.375</td>
<td>0.63</td>
</tr>
<tr>
<td>0.75</td>
<td>0.88</td>
</tr>
</tbody>
</table>

\[
\text{DCI} = \frac{(0.63-0.319)}{(0.88-0.63)} = 1.224
\]

\[
\text{Apparent Flux} = \frac{(0.88-0.319)}{0.75} = 0.748 \ \mu\text{L} \ \mathrm{N}_2\mathrm{O} \ \text{N} \ \text{L}^{-1} \ \text{h}^{-1}
\]

**Example Data Set 2**: \(\mathrm{N}_2\mathrm{O}\) time course chamber data.

<table>
<thead>
<tr>
<th>Headspace</th>
<th>(\mu\text{L} \ \mathrm{N}_2\mathrm{O} \ \text{L}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.319</td>
</tr>
<tr>
<td>0.375</td>
<td>0.425</td>
</tr>
<tr>
<td>0.75</td>
<td>0.513</td>
</tr>
</tbody>
</table>

\[
\text{DCI} = \frac{(0.425-0.319)}{(0.513-0.425)} = 1.20
\]

\[
\text{Apparent Flux} = \frac{(0.513-0.319)}{0.75} = 0.259 \ \mu\text{L} \ \mathrm{N}_2\mathrm{O} \ \text{L}^{-1} \ \text{h}^{-1}
\]
be 0.748 μL N₂O L⁻¹. If the GC precision is 2%, then this point (1.244, 0.748) is plotted on Fig 5. (shown as a red square). It is observed that the red point lies above the curve in Fig. 5, thus linear regression should not be used. The HM calculated flux for example 1 data is 0.923 μL N₂O L⁻¹ h⁻¹. In the example data set #2, the calculated DCI is 1.20 and the apparent flux is 0.259 μL N₂O L⁻¹ h⁻¹. If the GC precision happened to be 5%, then this point (1.20, 0.259) is plotted on Fig 6 (red square). Since this point falls below the curve linear regression would be the method of choice over the HM method. The decision criteria curves shown in Figs 5 and 6 are only for linear regression vs. HM method with 3 timepoint data sets. Families of decision criteria curves are currently being generated for the Quad and HM methods for 3 and 4 point rate data with GC precisions in the range of 1% to 6% (Parkin and Venterea, manuscript in preparation). These curves will be added to this document when they become available. Once the precision of a given GC system is known and its associated decision criteria curve has been generated, it should be relatively straightforward to code the selection of the best flux-calculation technique into a spreadsheet-based calculation system using conditional (If/Then) statements applied to each individual set of chamber data. This will require developing an empirical functional relationship between DCI and apparent flux (Parkin and Venterea, manuscript in preparation).

**Bias corrections and soil property effects:** Linear regression, and to a lesser extent the HM and Quad flux models, will generate negatively biased flux estimates even if correlation of the chamber data with the models are very high (Livingston et al., 2006; Venterea et al., 2009). Thus, fluxes estimated above are still expected to underestimate the actual pre-deployment soil-to-atmosphere fluxes. Additionally, the degree of bias and the extent of data curvi-linearity will increase with increased air-filled porosity in the soil underneath the chamber. This phenomenon occurs because as trace gas accumulates in the chamber following chamber closure, trace gas also accumulates in the air-filled soil pores, and a greater proportion of the total emitted trace gas will accumulate in the soil pores as the air-filled porosity increases (Venterea and Baker, 2008). This could result in important experimental artifacts, especially when soils under study differ with respect to bulk density and/or water content (for example when evaluating effects of tillage or organic amendments) (Fig. 7). This effect can also invalidate inter-site flux comparisons. In order to deal with these issues while avoiding the complications of using the NDFE model, Venterea (2010) developed a spreadsheet-based method for correcting the bias in flux estimates made using linear regression, HM, or Quad. This method accounts for the effects of soil properties, and therefore requires information regarding soil bulk density, water content, texture, and temperature at the time of flux-measurement. Measurement or estimate of these factors will necessarily introduce additional potential errors, but researchers wishing to make such

**Converting from Volumetric Units to Mass Units:** A flux calculated from either from linear regression or a non-linear model will have units of µL trace gas m\(^{-2}\) h\(^{-1}\) (when ppm(v) is regressed against time in hours). An additional calculation must be performed in order to covert flux values from a volumetric basis to a mass basis. To perform this conversion the ideal gas law is used (Eq. 4).

\[
P V = nRT
\]

Eq. 4

where \( P \) = pressure, \( V \) = volume, \( n \) = the number of moles of gas, \( R \) = the gas law constant, and \( T \) = temperature.

**Sample Calculation:** *Altitude = 1000 feet; Air temperature = 20°C*

To convert µL gas to µMol (value of 0.965 atm was obtained from Table 1 and °C was converted to °K by adding 273).

\[
1 \text{ µL trace gas} \times 0.965 \text{ atm} / ((0.08206 \text{ L atm Mol}^{-1} \text{ °K}^{-1}) \times (273 + 20)°\text{K}) \times 1 \text{ L/10}^6 \text{ µL} \times 10^6 \text{ µMol/Mol} = 0.0401 \text{ µMol trace gas}
\]

The ideal gas law quantifies the relationship between pressure, volume, mass and temperature of a gas. The ideal gas law constant (\( R \)) can be expressed in many different forms, but when \( R = 0.08206 \), the units are L atm Mol\(^{-1}\) °K. The corresponding units of \( P \), \( V \), \( N \) and \( T \) are atmospheres, liters, moles, and degrees Kelvin, respectively. The goal of applying Eq. 4 is to convert µL trace gas to µMol trace gas. To do this, one must have knowledge of both the air temperature and atmospheric pressure. Table 1 shows atmospheric pressures at different elevations. With knowledge of the temperature and altitude the ideal gas law is applied to convert µL of the trace gas to µMol of trace gas. For example, at an altitude of 1000 ft., and at an air temperature of 20°C, we calculate from Eq. 4 that 1µL of trace gas contains 0.0401 µMol.

<table>
<thead>
<tr>
<th>Alt (ft)</th>
<th>mm Hg</th>
<th>psi</th>
<th>atm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>29.92</td>
<td>14.7</td>
<td>1.000335</td>
</tr>
<tr>
<td>1000</td>
<td>28.86</td>
<td>14.18</td>
<td>0.964949</td>
</tr>
<tr>
<td>1320</td>
<td>28.54</td>
<td>14.02</td>
<td>0.954061</td>
</tr>
<tr>
<td>2000</td>
<td>27.82</td>
<td>13.67</td>
<td>0.930244</td>
</tr>
<tr>
<td>2640</td>
<td>27.14</td>
<td>13.33</td>
<td>0.907107</td>
</tr>
<tr>
<td>3000</td>
<td>26.81</td>
<td>13.17</td>
<td>0.896219</td>
</tr>
<tr>
<td>3960</td>
<td>25.77</td>
<td>12.66</td>
<td>0.861513</td>
</tr>
<tr>
<td>4000</td>
<td>25.84</td>
<td>12.69</td>
<td>0.863555</td>
</tr>
<tr>
<td>5000</td>
<td>24.89</td>
<td>12.22</td>
<td>0.831571</td>
</tr>
<tr>
<td>5280</td>
<td>24.47</td>
<td>12.02</td>
<td>0.817961</td>
</tr>
<tr>
<td>6000</td>
<td>23.98</td>
<td>11.78</td>
<td>0.801629</td>
</tr>
<tr>
<td>6600</td>
<td>23.25</td>
<td>11.42</td>
<td>0.777131</td>
</tr>
<tr>
<td>7000</td>
<td>23.09</td>
<td>11.34</td>
<td>0.771687</td>
</tr>
<tr>
<td>7920</td>
<td>22.15</td>
<td>10.88</td>
<td>0.740384</td>
</tr>
<tr>
<td>8000</td>
<td>22.22</td>
<td>10.91</td>
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</tr>
<tr>
<td>10560</td>
<td>20.11</td>
<td>9.88</td>
<td>0.672334</td>
</tr>
</tbody>
</table>
μMol of trace gas (see sample calculation box). Thus, 1 ppm (1 μL/L) trace gas contains 0.0401 μMol trace gas per L of air. If temperature is changing significantly during chamber deployment (by more than about 5°C per hr), and temperature corrections per above are not applied, this will cause errors in calculated fluxes due to expansion (temperature increase) or contraction (temperature decrease) of chamber headspace gas.

**Minimum detection limit and non-significant fluxes:** Often field fluxes are low, thus it is important to have an idea of the minimum detection limit (MDL). To determine the MDL we performed Monte Carlo simulations over a range of analytical precisions and chamber deployment times (Parkin and Venterea, manuscript in preparation). Results of these studies for N2O are presented in Tables 2 and 3 for 3-point and 4-point data sets, respectively. The limits presented in Tables 2 and 3 are for positive fluxes. The detection limits for negative fluxes can be obtained by multiplying the values in Tables 2 and 3 by -1. These “negative” MDLs will then represent the upper limit for gas consumption processes which are manifested as negative fluxes. There are several options available to handle data that falls below the MDL (or within the detection limit band). These options include: 1) report the value as “below the detection limit”, 2) report the value as zero, 3) report some a value between zero and the MDL (such as ½ the MDL), or 4) report the actual measured value even if it falls below the MDL (Gilbert, 1987). **We recommend that, in reporting trace gas studies in this project, option 4 be adopted - report the measured value along with the stated MDL.**

Table 2. Minimum Detection Limits (α = 5%) for Hutchison/Mosier (H/M) procedure the Quadratic procedure (Quad) and linear regression (L.R.) for different chamber deployment times. Three equi-spaced data points for each flux determination.

<table>
<thead>
<tr>
<th>Analytical Precision (%CV)</th>
<th>0.5 Hour Deployment</th>
<th>0.75 Hour Deployment</th>
<th>1.0 Hour Deployment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HM</td>
<td>Quad</td>
<td>LR</td>
</tr>
<tr>
<td>1</td>
<td>34.6</td>
<td>64.1</td>
<td>17.7</td>
</tr>
<tr>
<td>2</td>
<td>70</td>
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<tr>
<td>3</td>
<td>105</td>
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<td>53.3</td>
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<td>4</td>
<td>140</td>
<td>258</td>
<td>71.1</td>
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<tr>
<td>5</td>
<td>176</td>
<td>322</td>
<td>88.9</td>
</tr>
<tr>
<td>6</td>
<td>213</td>
<td>387</td>
<td>107</td>
</tr>
</tbody>
</table>
Table 3. Minimum Detection Limits ($\alpha = 5\%$) for Hutchison/Mosier (H/M) procedure the Quadratic procedure (Quad) and linear regression (L.R.) for different chamber deployment times. Results are for 15,000 Monte Carlo simulations at each analytical precision and deployment time. Four equi-spaced data points for each flux determination ($t_0, t_1, t_2, t_3$). For the H/M flux calculations the average concentration of the $t_1$ and $t_2$ data points was used.

<table>
<thead>
<tr>
<th>Analytical Precision (%CV)</th>
<th>0.5 Hour Deployment</th>
<th>0.75 Hour Deployment</th>
<th>1.0 Hour Deployment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HM</td>
<td>Quad</td>
<td>LR</td>
</tr>
<tr>
<td>1</td>
<td>21.2</td>
<td>47.0</td>
<td>16.9</td>
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<tr>
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<td>42.3</td>
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<tr>
<td>3</td>
<td>62.7</td>
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<tr>
<td>4</td>
<td>84.4</td>
<td>186</td>
<td>67.3</td>
</tr>
<tr>
<td>5</td>
<td>105</td>
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<td>84.5</td>
</tr>
<tr>
<td>6</td>
<td>126</td>
<td>278</td>
<td>101</td>
</tr>
</tbody>
</table>

Non-Detects: As was noted above, analytical and sampling error introduces variability that can result in “noisy” data. Fig. 8 shows the 17 possible data patterns that can be obtained from 3 point sample sets. One consequence of noisy data is that the HM model will often not be applicable. The HM model will only work if the quantity $[(C_1 - C_0)/(C_2 - C_1)] > 1$ (Fig. 8, panels 2 and 3) or the quantity $[(C_1 - C_0)/(C_2 - C_1)]$ is between zero and 1 (Fig. 8, panels 8 and 9). In 13 of the 17 possible data patterns shown in Fig. 8, the HM model will fail. In cases of HM failures, the investigator can: 1) designate the flux as zero, 2) use linear regression, or 3) use an alternate method (i.e. Quad, NDFE, HRM). Until the non-linear models (NDFE and HMR) can be further evaluated, at this point in time we recommend that the investigator use linear regression (option 2) or the Quad method when the HM model fails. Often an outlier may be present, due to sampling or analytical problems (i.e. vial leakage, chamber leakage, sample mix up, or change in GC detector sensitivity, humidity or temperature perturbations). Critical judgment is required to
disregard outliers. If N$_2$O, and CO$_2$ analyses are performed on the same sample (N$_2$O with an electron capture detector and CO$_2$ with a thermal conductivity or methanizer + flame ionization detector or infra-red detector), then often a sampling or analytical problem can be diagnosed by comparing N$_2$O and CO$_2$ data for each timepoint. For example, if data pattern 4 or 5 (Fig. 8) is observed for both N$_2$O and CO$_2$, a likely explanation would be chamber or sample vial leakage, since, in opaque chambers, consumption of atmospheric CO$_2$ is not typical. Similarly, if CO$_2$ data patterns like those of panels 7, 8, or 9 were observed and the CO$_2$ concentration of last time point were near ambient, this may indicate a sample mix up (i.e. $t_0$ exchanged with $t_2$). Temperature or humidity changes during chamber deployment may produce patterns similar to those of panels 4, 5, and 6. If the investigator cannot discount outliers based on experience and judgment of past performance of the site, instrument function, or chamber efficacy, the most conservative approach would be to use linear regression on all the data. If noisy data proves to be a persistent problem, evaluation of GC precision, chamber design, septa reactivity/integrity, and sampling protocols should be performed. Also, collection of 4 (or more) gas samples during the chamber deployment will yield improved flux estimates.

Quality assurance /Quality control:
Standards and standardization: Standards should be prepared with each set of samples. Linearity of the detector’s responses should not be assumed, thus a range of gas concentrations for each trace gas be run. Standard gasses should be prepared in an “air matrix” unless it has been previously determined that GC detector response is not sensitive to O$_2$. GC drift can occur during a run, thus it is recommended that check standards be run every 10 or 20 samples to determine, and if necessary, correct for GC drift. It is highly recommended that a NIST-certified tank containing CO$_2$, N$_2$O and CH$_4$ be purchased by the project and used to evaluate the standard gases used at all locations.

Ancillary Measurements
In addition to the measurements prescribed by soil sampling protocol additional measurements are recommended.

1. *At the times fluxes are measured:* 1) air temperature, 2) soil temperature (5 cm), and 3) soil water content (0-6 cm).
2. *At the time of chamber anchor installation:* 1) bulk density, 2) soil texture, 3) organic C and N., 4) pH, 5) anchor height above the soil (used to compute chamber headspace volume), 6) soil nitrate and ammonium (0-10 cm). [Note: It is desirable that soil nitrate and ammonium be determined throughout the year at time intervals deemed appropriate by the individual investigator as dictated by resource availability and plot constraints.]
3. *Year round:* The following meteorological should be collected year round at a frequency of at least once per day: 1) precipitation, 2) air temperature, 3) relative humidity, and 4) solar radiation.
Advice and Consultation
Several USDA-ARS investigators involved in GRACEnet have experience in trace gas analysis and flux measurement. These people have agreed to serve as resource contacts for investigators with questions on GC operation, soils chambers, gas sampling, flux calculation, field variability, and data calculations and interpretation.

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Literature Cited


Hutchinson, G.L. and G.P. Livingston. 1993. Use of chamber systems to measure trace gas fluxes. In L. Harper et al. (eds) Agricultural Ecosystem Effects on Trace Gases and Global Climate Change, pp. 63-78. ASA Special Publication 55, ASA, CSSA, SSSA, Madison, WI.


Smith, K. A. And K.E. Dobbie. 2001. The impact of sampling frequency and sampling times on chamber-based measurements of N2O emissions from fertilized soils. Global Change Biol. 7:933-945.
Other Useful References


Appendix I. Example of Trace gas Flux Sampling Procedure
A set of 12 Anchors placed in pairs (in-row and inter-row). For each set of 12 chambers:
1. Lay out Chambers, Vials, Syringes by each anchor
2. Install 5 cm temperature Probes (1 in each plot). Air temperature and chamber temperature probes are placed in first plot only.
3. Take ambient air gas sample
4. Start Measurement (t 0)
a. Start at plot #1
   1. Record Temperatures, Start Stop Watch
   2. Place chamber on anchor #1 (vent facing downwind)
   3. Remove 10 ml gas sample
   4. Inject sample into vial
   5. Flush syringe with Air 2x
   6. Place chamber on anchor #2
   7. Remove 10 ml gas sample
   8. Inject sample into vial
   9. Flush syringe with air 2x
b. Move to the pair of chambers in the next plot
   1. Record time on stop watch
   2. Place chamber 3 on anchor
   3. Remove 10 ml gas sample
   4. Inject into vial
   5. Flush syringe with Air 2x
   6. Place chamber 4 on anchor
   7. Remove 10 ml gas sample
   8. Inject into vial
   9. Flush syringe with air 2x
c. Move to next plot
   2. Repeat steps 4b.1 through 4b.9 (above)
d. Repeat step 4c until all 12 chambers are in place and have been sampled for time 0
5. First Time Point (t 1)
a. Move to plot #1 (chamber 1)
   1. Record Soil Temperatures, record chamber temperature and air temperature.
   2. Insert syringe into chamber septa
   3. When stopwatch shows t-1 time (e.g. 20 minutes), remove 10 ml Gas sample
   4. Inject gas sample into appropriate vial
   5. Flush syringe 2x
   6. Move to next chamber, repeat steps 5a.2 - 5a.5, above.
   7. Continue until all chambers have been sampled for time 1
5. Second and third time points (t 2 and t-3)
a. same as step 5 above.
6. Remove all chambers, Move to next set of 12 anchors. Repeat steps 1-5
7. When all plots have been done, one person collect all chambers and place in truck other person take soil moisture readings in each plot (4 measurements/plot).
Appendix II: Potential Vendors for Supplies*

Sample Vials and Stoppers:
Option 1. Exetainer vials, screw cap 12 ml vials that have a butyl rubber septa-same idea as the serum vials and butyl rubber stoppers-just cheaper and more or less disposable-can buy new screw caps and septa relatively cheaply. Exectainer vials are purchased through Labco Limited (Brow Works, Copyground Land, High Wycombe, Buckinghamshire. HP123HE, United Kingdom (phone 44-1494-459741) (fax: 44-1494-465101) (Email: sales@labco.co.uk or enquiries@labco.co.uk).

Option 2. Glass serum vials 6.0 ml (22 x 38 mm) Alltech, 2051 Waukegan Rd, Deerfield, IL 60015 (vial stock # 98768)
Butyl rubber stoppers; 20 mm round bottom (part # 73828A-RB). Aluminum crimps (20 mm); (part # CTO20NAT), Voigt Global Distribution, Inc. P.O. Box 1130, Lawrence, KS 66044. 877-484-3552. www.vial-seals.com


Syringe stopcocks: www.coleparmer.com

Reflective Mylar Tape or insulation: www.uline.com, www.reflectix.com


*Reference to a trade or company name is for specific information only and does not imply approval or recommendation of the company or product by the U.S. Department of Agriculture (USDA) to the exclusion of others that may be suitable.
Appendix III. Examples of some chambers.

PVC soil anchor and chamber used by Hutchinson and Mosier.
Rectangular Chambers used at Ft. Collins, CO Location.
Gas sample collection from chambers at Ames, IA location. Thermometers are measuring air and chamber headspace temperatures.
Example of temporary/portable chamber used by Parkin et al, (2005). Chamber has an attached polyethylene skirt held in place on the soil surface with a length of chain. As shown, the chamber is monitoring soil CO$_2$ flux by recirculating gas through an infrared analyzer. Gas samples can be withdrawn through septum in top of chamber for N$_2$O and CH$_4$ analyses.
Stainless steel chamber tops (above) and anchors (below) used in St. Paul, MN. Chamber anchors are nearly flush with soil surface. See Appendix IV for construction details. Photos on following page shows inside of chamber top and sample collection method.
Appendix IV. A. Example of circular chamber construction used at the Ames, IA location.

CHAMBER CONSTRUCTION

Cut a 10 cm length of PVC pipe for the chamber top and a 15 cm length for the ring that will serve as the chamber anchor. Using a router with a 45 degree bevel chamfer bit, make a reasonably sharp edge on the anchor PVC ring. This will make it easier to insert into the soil.

The 10 cm long PVC ring will be used for the lid. Make a threaded 7/16” hole about 1” in from the edge of the ring. This side will be the top of the lid.

Trace the outside of the ring onto the PVC sheet (1/4 “ thick) and cut out this circle. Apply PVC primer to the outside of this circle and to the thin edge of the ring. When dry, apply the cement to those same areas and attach the PVC circle to the top of the lid. Weigh it down with something to get a good seal.

After the cement has set, drill a ½” hole in the PVC circle approximately halfway between the center of the circle and the outside edge. The 20 mm butyl rubber stopper will go in here.

Attach a 15 cm piece of SS tubing to the straight union fitting and screw the fitting into the threaded hole in the ring. This is the chamber vent and will be inside the lid.

Cut an approximate 7 cm wide strip of the tire tube(make sure this is cut so as to get one continuous piece). Put this around the bottom of the lid. It will fit very snugly. Half of the tube strip will be on the lid and half will be hanging off the bottom. Now tape the rubber strip, which is on the lid, to the outside of the lid using the duct tape.

Put overlapping strips of reflective mylar tape on the chamber, top and side, so that it is nearly totally covered on the outside. Fold over the rubber strip so that the edge of the ring is showing. On this edge of the ring, the weather strip will go.

Place a thin layer of contact cement on the edge of the chamber. When the cement becomes tacky, apply the weather strip.

Place the butyl rubber stopper in the chamber tip and secure with duct tape.

Photographs of construction details are shown below.

MATERIALS LIST

PVC pipe, 12” diameter, schedule 40
Straight union fittings, ¼” PP
Tractor tire tube, 15.5R38
PVC sheet, grey, ¼" thick, grade 1 type 1
Metalized Mylar Film tape, 2" width, silver
PVC Purple Primer
PVC cement
Rubber Weatherseal, 3/8" wide x ¼" thick, “D” profile
Stainless Steel Tubing, ¼"
20mm Butyl Rubber Stoppers
Duct Tape
Fig. 1. PVC ring 30.3 cm inner diameter, 10 cm long.

Fig. 2. Circle cut from ¼” thick PVC sheet.

Fig. 3. PVC circle glued to PVC ring.

Fig. 4. Strip cut from truck tire inner tube (7 cm wide).

Fig. 5. Inner tube strip placed on PVC ring.

Fig. 6. Inner tube strip on PVC ring.

Fig. 7. Inner tube strip is taped into place.

Fig. 8. Inner tube strip folded back onto PVC ring.

Fig. 9. Thin layer of contact cement applied to PVC edge.

Fig. 10. Weather strip to be applied to PVC edge.

Fig. 11. Weather strip is first separated to single strand.

Fig. 12. Weather strip being applied to edge of PVC ring.
<table>
<thead>
<tr>
<th>Fig. 13. Edges of weather strip are sealed with glue.</th>
<th>Fig. 14. Hole is drilled in side of chamber top.</th>
<th>Fig. 15. Hole is tapped to accept vent tube fitting.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig. 16. Plastic union serves as vent tube fitting.</td>
<td>Fig. 17. Plastic union screwed into hole in chamber top.</td>
<td>Fig. 18. Stainless steel (1/4&quot;) is attached to plastic union.</td>
</tr>
<tr>
<td>Fig. 19. Hole drilled in top for sampling septa.</td>
<td>Fig. 20. Reflective mylar tape applied to chamber side.</td>
<td>Fig. 21. Reflective mylar tape applied to chamber top.</td>
</tr>
<tr>
<td>Fig. 22. Butyl rubber septa placed in chamber top hole.</td>
<td>Fig. 22. Butyl rubber septa in top.</td>
<td>Fig. 23. Septa held in place with tape.</td>
</tr>
</tbody>
</table>
Fig. 24. PVC anchor ring (15 cm), one edge beveled.

Fig. 25. Chamber is being placed on anchor ring.

Fig. 25. Chamber on anchor – inner tube is folded up.

Fig. 26. Inner tube being folded down onto anchor.

Fig. 27. Inner tube being folded onto anchor.

Fig. 27. Chamber in place on anchor.

Fig. 28. Chamber should have tight fit to anchor.
B. Schematics for rectangular chamber construction used at the Ft. Collins, CO Location.

**Rectangular aluminum Chambers:** Made from sheet aluminum. These can be made any size to fit the field situation.

Anchors: Made from sheet aluminum with a trough to hold water that has been welded on top. The anchors are inserted 10 cm into the soil.

Chamber: Made from sheet aluminum to desired dimensions. Two holes, to accommodate Swagelock fittings for vent tube and gas collection septum are drilled and tapped in each chamber top.
FRAME OF CHAMBER MADE FROM 1 INCH ALUMINUM CHANNEL TUBING: REQUIRES WATER-TIGHT WELDS

43 CM

38 CM

75 1/2 CM

80 1/2 CM

SILICONE SEAM TO MAKE AIRTIGHT

10 CM

ALUMINUM STRIP 1/8" THICK WELDED INSIDE FRAME

DRAWING 2 OF 2

TRACE GAS ANCHOR  3/32" = 1 CM

USDA-ARS-SPNR  3-2003
Description of construction for stainless steel low-profile chambers used at St. Paul, MN ARS location (see photos in Appendix III)

Chamber anchors and tops are fabricated using 20-gauge rectangular stainless steel “steam pans” equipped with a flange around the edges (Superior Products, St. Paul, MN). Anchors are made by cutting out the bottom section of the pan resulting in a frame measuring 0.50 m X 0.29 m X 0.086 m deep, which is inserted into the soil so that the flange is nearly flush with the soil surface. Chamber tops (0.50 m X 0.29 m X 0.102 m high) are further fabricated by attaching weather-stripping material (EPDM) to the flange to serve as a gasket, covering the outer surfaces with reflective insulation (Reflectix, Markleville, IN), and installing a vent tube (3.5 mm ID X 0.15 m long) horizontally on one side and a septum-lined sampling port in the top. The sampling port is connected on the inside of the chamber to a manifold (Part no. STCM-13-20/4, Small Parts, Inc., Miramar, FL) which in turn was connected to 4 sections of FEP tubing (0.8-mm ID X 0.2 m long) (Cole Parmer) with one section of tubing secured in each quadrant of the chamber. Additional details including additional photographs and links to material suppliers can be found on-line at:

http://www.ars.usda.gov/pandp/docs.htm?docid=19008
Video Article

Measurement of Greenhouse Gas Flux from Agricultural Soils Using Static Chambers

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Keywords: Environmental Sciences, Issue 90, greenhouse gas, trace gas, gas flux, static chamber, soil, field, agriculture, climate

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Abstract

Measurement of greenhouse gas (GHG) fluxes between the soil and the atmosphere, in both managed and unmanaged ecosystems, is critical to understanding the biogeochemical drivers of climate change and to the development and evaluation of GHG mitigation strategies based on modulation of landscape management practices. The static chamber-based method described here is based on trapping gases emitted from the soil surface within a chamber and collecting samples from the chamber headspace at regular intervals for analysis by gas chromatography. Change in gas concentration over time is used to calculate flux. This method can be utilized to measure landscape-based flux of carbon dioxide, nitrous oxide, and methane, and to estimate differences between treatments or explore system dynamics over seasons or years. Infrastructure requirements are modest, but a comprehensive experimental design is essential. This method is easily deployed in the field, conforms to established guidelines, and produces data suitable to large-scale GHG emissions studies.

Introduction

Understanding the contributions of both human activities and natural systems to radiative properties of the atmosphere is an area of critical importance as we strive to mitigate anthropogenic contributions to the greenhouse effect. In addition to carbon dioxide (CO₂), nitrous oxide (N₂O) and methane (CH₄) are also potent GHGs, accounting for an estimated 7% and 19% of global warming, respectively, with the majority of emissions coming from landscape sources²,⁵. These range from managed systems such as agricultural fields, rice paddies, and landfills, to natural systems such as forest floors, wetlands, and termite mounds. Accurate measurement, supporting well-informed modeling of such landscape-based emissions is critical in order to understand the drivers of climate change as well as to identify mitigation opportunities.

A variety of greenhouse gas measurement strategies exist, each with their own strengths and weaknesses⁵,⁶. Mass balance techniques rely on wind-based dispersion of gases and are suited to measurement of flux from small, well-defined sources such as landfills and animal paddocks. Micrometeorological approaches such as eddy covariance are based on real-time direct measurement of vertical gas flux, and can provide direct measurements over large areas. However, homogeneity in source topography is an implicit assumption (in that measurements yield a mean for the area under study), and costly infrastructure can limit deployment possibilities. Finally, chamber-based methods focus on change in gas concentration at the soil surface by sampling from a restricted above ground headspace. They allow measurements to be obtained from small areas and numerous treatments, but are subject to high coefficients of variation due to spatial variation in soil gas flux.

Here we discuss the most prevalent and easily implemented form of chamber-based measurement, utilizing the type of closed chambers without air flow-through commonly referred to as “static” or “non-steady-state non-flow-through” chambers. In this approach, gas emissions from the soil surface are trapped within a vented chamber, and rates of flux are determined by measuring the change in gas concentration over time within the chamber headspace. The static chamber technique has been widely deployed across both managed and natural landscapes and underpins the surface are trapped within a vented chamber, and rates of flux are determined by measuring the change in gas concentration over time within the chamber headspace. The static chamber technique has been widely deployed across both managed and natural landscapes and underpins the

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Change in gas concentration over time is used to calculate flux. This method can be utilized to measure landscape-based flux of carbon dioxide, nitrous oxide, and methane, and to estimate differences between treatments or explore system dynamics over seasons or years. Infrastructure requirements are modest, but a comprehensive experimental design is essential. This method is easily deployed in the field, conforms to established guidelines, and produces data suitable to large-scale GHG emissions studies.

The video component of this article can be found at http://www.jove.com/video/52110/
As a critical tool in GHG measurement and flux estimation, the static chamber method has been thoroughly evaluated, and significant efforts have been made towards standardization of techniques and harmonization of data reporting. Of particular note are the detailed reviews and guidelines produced by the U.S. Department of Agriculture – Agricultural Research Service’s Greenhouse gas Reduction through Agricultural Carbon Enhanced network (GRACEnet) and by the Global Research Alliance on Agricultural Greenhouse Gases (GRA). Such guidelines provide an invaluable resource and platform for coordination, as ultimately the interoperability of data from a myriad of studies is critical for scaling up local findings to global modeling, and for translating research results into viable mitigation strategies.

GRACEnet, GRA, and other reviews also highlight the fact that specific techniques in static chamber-based greenhouse gas flux measurement are extremely diverse, with significant methodological variations possible at nearly every step of the way, including chamber design, temporal and spatial deployment, sampling volumes, sample analysis, and flux calculations. The method described here presents one possible variant, while showcasing best practices and highlighting critical considerations for the generation of high quality, broadly transferable data. It is intended to provide an accessible overview of this standardized procedure, and a platform from which to explore further nuances and variations described in the literature.

### Protocol

#### 1. Chamber Construction and Anchor Installation

1. Design and construct chambers – each consisting of an anchor that is inserted into the soil and a lid that is placed on top of the anchor during flux measurement – to meet experimental needs.
   1. In designing chamber shape and size, consider spatial factors such as crop row spacing, fertilizer or manure banding, and plant height. Because protrusion of anchors above the soil surface can contribute to microclimate effects and water ponding, consider having the lids sit as low to the soil surface as possible. Because tradeoffs exist between chamber height and detection sensitivity, design lids to be as short as is feasible for the system under study.
   2. Build chambers of sturdy, nonreactive material such as stainless steel or PVC, and include a mechanism for sealing the lid onto the anchor. Insulate lids and cover with light-colored or reflective material to prevent heat buildup during measurement. Include a septum to allow sample collection and a vent tube to prevent pressure perturbations during chamber deployment and sample removal. For additional details refer to the Materials table, Parkin and Venterea, and Clough et al.

2. At least 1 day prior to sampling, install chamber anchors in the soil at desired sites. The installation method will depend on chamber design, but in general, apply even pressure across all points so that the anchor does not warp or distort the soil structure. Sink the anchor to a depth of 2.5-13 cm depending on soil type, deployment time, and chamber volume. Leave as little as possible (no more than 5 cm) protruding above the soil surface.

#### 2. Calibration and Experimental Design

Note: Prior to beginning the experiment, follow these steps to determine an appropriate sampling time course that will allow data to be fit to an appropriate linear or non-linear flux model (see Parkin et al.). This will require the use of techniques described in steps 3-5 (Field Sampling, Sample Analysis, and Data Analysis). Optimal timing is a function of both the system under study and the dimensions of chambers being used. Some trial and error may be involved. See Venterea for alternate approaches.

1. Calibration Sampling and Analysis
   1. Under environmental or management conditions expected to generate relatively high trace gas fluxes, conduct intensive sampling following techniques described in section 3. Using tightly spaced sampling time points, populate a time series of longer duration than would be considered typical. Begin by sampling from several representative chambers at 5-10 evenly spaced time points over the course of an hour.
   2. Analyze samples by gas chromatography following section 4.

2. Calibration Interpretation
   1. For each calibration time series and each gas of interest, plot time-by-concentration.
   2. Verify that flux rates are on the high end of the expected range. See section 5 for flux calculation. Refer to section 2.3 for troubleshooting tips.
   3. Inspect graphs for signs of non-linearity, or more specifically, plateauing of gas concentrations over time.
   
   Note: The point at which concentration begins to plateau differs by gas type, and is a function of the rate of gas production or consumption within the soil, the concentration of the gas in the chamber headspace, and diffusion between the two zones. It is therefore strongly affected by chamber height, with shorter chambers yielding shorter time before plateau.
   4. Use the calibration sets to determine optimal chamber deployment time for the experimental system. If linear regression will be used in data analysis (as described here in section 5), select timing that maintains as close to a linear relationship as possible between time and concentration for all gases / systems of interest, while allowing for a minimum of three, preferably four, sampling times within the time series. For chambers 10-30 cm high used for CO₂ and N₂O measurements, time series typically range from 20-60 min.

3. Calibration Troubleshooting
   1. If there is poor differentiation and/or difficulty discerning linearity or plateau, use tighter calibration time points or longer calibration time series, and check that concentrations are within detection limits. For low rates of flux, a reduction in rate of accumulation may not be observed within the tested timeframe. This should not cause concern.
   1. If the fluxes are not at the high end of the expected experimental range, repeat calibration, altering treatment or environmental conditions to induce higher flux (by applying fertilizer or irrigation, for example). Alternately, use at least four time points in experimental design, so that if experimental fluxes are significantly higher than those observed during calibration and plateauing does occur, later
time points can be excluded while retaining at least three time points for linear regression. Curvilinear regression approaches may also be employed.

4. Experimental Design
   1. Based on the optimal timing determined in section 2.2.4, devise an overall sampling scheme that captures all relevant sites, treatments, and/or replicates, and allows personnel to move through chamber sites efficiently. If necessary, divide the chamber sites into several “rounds” to be sampled one after the other.
      1. If measurements are to be taken as representative of a whole day, sample at a time of day when temperatures are moderate relative to daily extremes. In typical temperate cropping systems, the ideal window is mid- to late-morning.
      2. If samples are to be collected in consecutive rounds, be careful not to introduce a bias by repeatedly sampling the same treatments at the same time of day. Construct rounds out of blocks of replicates rather than treatment-by-treatment.
      3. Include time for any necessary ancillary measures to be taken either within rounds or before/after, as appropriate. (See section 3.3 for typical ancillary measures.)
   2. Determine the frequency of flux measurements that is appropriate for research goals. This may range from a single measurement to daily, weekly, or periodic measurements over the course of months or years. Refer to Rochette et al.\textsuperscript{14} for a thorough discussion of experimental design considerations.
   3. If samples are to be collected in cold conditions, plan for inclusion of a warming device such as a hot pack with vials to prevent septa from becoming brittle.

3. Field Sampling

Note: On each sampling date, follow the sampling scheme established in section 2.4, using the techniques described below. Equipment and sample volume can vary depending on the collection and transfer methods being employed and the amount of sample required for GC analysis\textsuperscript{8}. This protocol utilizes 5.9 ml collection vials and 30 ml syringes, with a flushing method of sample transfer. See Discussion for alternate approaches.

1. Preparation
   1. If sampling from multiple chambers per round, prepare a time point reference grid (see Figure 2) to easily track where and when to sample. Alternately, make arrangements to record each time point during sampling.
   2. Pre-label and arrange collection vials for maximal efficiency and minimal likelihood of confusion during sampling.
   3. In order to save time during sampling, prepare all materials and equipment beforehand. Include extras of anything that may break or is easily lost (needles, syringes, stopcocks, etc.), and place in a carry tote, bucket, or other container.
   4. Be prepared to record any delayed time points which can happen due to equipment malfunction or other unforeseen circumstances, and which can be easily corrected during data analysis by adjusting the time associated with a certain sample.

2. Sample Collection
   1. Attach and seal the chamber lid to the pre-installed chamber anchor, and start a stopwatch. This is $T_0$.
   2. Immediately after sealing the lid collect a sample of ambient air from a location adjacent to the chamber, at the approximate height of the chamber top: with an empty 30 ml syringe fitted with a needle and a stopcock in the open position, draw a 30 ml air sample and close the stopcock. This is the $T_0$ sample. Alternatively, take the $T_0$ sample from the chamber.\textsuperscript{6}
   
   Note: Tradeoffs exist between the two approaches – evaluate spatial (distance from site or external microclimate for outside samples) vs. timing (delay between lid closure and sample collection for inside samples) considerations and determine the most appropriate technique for the equipment being used and the system under study.

   3. With the syringe needle, pierce the septum of a 5.9 ml collection vial that already has another needle poked through near the edge of the septum.
   4. Open the syringe stopcock and inject approximately 20 ml of the sample into the vial (this causes the previous contents of the vial to be expelled through the extra needle, replaced by sample).
   5. In a smooth motion, remove the extra needle while continuing to inject as much of the remaining sample (approximately 10 ml) as possible, slightly over-pressurizing the vial to ensure sample integrity and allow analysis of multiple samples if necessary.\textsuperscript{5}
   6. Close the stopcock and withdraw the syringe needle from the septum. Turn the filled vial upside-down to distinguish from unfilled vials.
   7. Proceed to the next chamber, repeat steps 3.2.1-3.2.6, sealing the lid on the correct pre-determined $T_0$ time point.
   8. Continue to repeat steps 3.2.1-3.2.7 until all chambers in the round have been sealed and $T_0$ samples have been collected.
   9. Return to the first chamber.
   10. As the time approaches 10 seconds until $T_1$, pierce the septum in the chamber top with the syringe needle.
   11. Within a 10 second range of $T_1$, withdraw a 30 ml sample of air from inside the chamber and close the stopcock. Remove the syringe needle from the chamber septum.
   12. Transfer the sample to a collection vial following steps 3.2.3-3.2.6.
   13. Continue to collect samples following steps 3.2.10-3.2.12, according to the sampling scheme established in section 2.4.

3. Ancillary Measures
   1. In order to convert gas concentration to mass, measure the air temperature at the time of sampling. Depending on research goals, record or perform other ancillary measures such as soil temperature and soil moisture content at each location and/or time, daily rainfall, soil bulk density, soil nitrate and ammonium concentrations, etc. Various means exist to obtain these measures – follow standard protocols.
2. Optionally, collect ambient air samples and/or load field standards of known concentrations into vials to assess ambient GHG concentrations and potential storage-vial degradation in the period between sampling and analysis (see sections 2.4.1.4 and 2.4.1.5).

4. Sample Analysis

1. Determine the concentration of gases of interest for each sample by gas chromatography, using equipment fitted with an electron capture detector for N\textsubscript{2}O, an infrared gas analyzer or thermal conductivity detector for CO\textsubscript{2}, and a flame ionization detector for CH\textsubscript{4}.

   Note: It is essential to obtain access to an instrument that is properly configured for GHG analysis and has sufficient run time available. Principles and methods of gas chromatography are described elsewhere\textsuperscript{6,15,16}.

2. Convert trace gas concentration from volumetric to mass using the Ideal Gas Law:

   \[ PV = nRT \]

   Where P = pressure, V = volume, n = moles of gas, R = gas law constant, and T = temperature. Thus:

   \[
   \frac{V \text{ trace gas L} \cdot 1 \text{ L}^{-1} \cdot P \text{ atm}}{(0.08206 \text{ L atm mol}^{-1} \cdot K^{-1}) \cdot (273 + T \cdot ^{\circ}C)^{-1} \cdot K}\]

5. Data Analysis

1. For each time series, plot time-by-concentration and evaluate for linearity. Evaluate using goodness of fit or by visual inspection, excluding later time points showing signs of plateau from further analysis. Use a minimum of three time points including T\textsubscript{0} for flux calculation (T\textsubscript{0}, T\textsubscript{1}, T\textsubscript{2}, ...). Establish a consistent protocol, and reject any time series that fail to meet that protocol's standards for linearity. See Parkin and Venterea\textsuperscript{8} for a thorough discussion of error, bias, and variance in flux calculation.

2. Perform linear regression.

3. Use the slope of the regression to calculate flux:

   \[ F = S \cdot V \cdot A^{-1} \]

   Where F = flux, S = slope of the regression, V = chamber volume, and A = chamber area. Thus:

   \[
   \frac{(S \text{ mol L}^{-1} \cdot hr^{-1}) \cdot V \text{ L}}{A \text{ m}^2}\]

   Note: Refer to the Discussion and Parkin \textit{et al.}\textsuperscript{12} for non-linear approaches to flux calculation.

Representative Results

Prior to beginning a research project with static chambers, it is important to understand the overall workflow, and the organization of \textit{in silico}, field- and laboratory-based elements (Figure 1). Provided careful experimental design and system calibration (Figure 2), data analysis will generally be relatively straightforward. A rate of flux is determined for each chamber and sampling time by regression of time by concentration using a pre-determined flux model appropriate to the system (Figure 3). However, even following best practices, difficulties may be encountered, and quality control of raw data is critical. For example, failure of a chamber seal or leaky sample vials can result in anomalous concentration values. These are readily identified through visual inspection of time series concentration plots (Figure 4), with CO\textsubscript{2} time series often serving as a particularly useful indicator due to the typically more robust and continuous flux of CO\textsubscript{2} compared to sometimes negligible, near-detection-limit, or even negative fluxes of N\textsubscript{2}O or CH\textsubscript{4}. Once data quality has been confirmed, results may be used to compare gas flux dynamics between treatments or over the course of a season (Figure 5). As can be seen from May and June flux values and error bars, the variation caused by spatial heterogeneity of flux may be significant, and more pronounced under conditions producing high rates of flux. Such variability is not unusual, and underscores the importance of sufficient replication in this technique.
Figure 1. Workflow overview. Various elements of this protocol will be carried out in the planning stage, in the field, in the laboratory, and in silico. Arrows indicate the sequence of workflow, beginning with chamber design (and construction if necessary), and concluding with data analysis. Multiple boxes/arrows between field sampling and sample analysis represent the possibility of multiple sampling dates over the course of an experiment.

<table>
<thead>
<tr>
<th>Chamber</th>
<th>T₀</th>
<th>T₁</th>
<th>T₂</th>
<th>T₃</th>
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<tr>
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<td>12</td>
<td>24</td>
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<td>4</td>
<td>9</td>
<td>21</td>
<td>33</td>
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</table>

Figure 2. Sample timing. An example timing scheme for the collection of samples from multiple chambers simultaneously. Chamber numbers are indicated at left and time points at top, with sampling times listed in whole minutes within the grid. In this example, four separate time series of 36 min each (one for each chamber) are carried out within the space of 46 min, with 12 min spacing between time points within a series, and 2 min walking time between chambers. For this hypothetical example, the suitability of 36-min time series would have been determined by prior calibration. While evenly spaced timing is not necessary, it often simplifies the sampling scheme. Alternately, researchers may individually record each sampling timepoint to determine sampling intervals.
Figure 3. Flux calculation. A typical static chamber time series, consisting of N₂O concentrations measured at four time points over the course of a 36-min sampling period. The linear regression is displayed, the slope of which yields flux rate.

\[
y = 0.0838x + 0.4245
\]

\[2.64 \text{ g N}_2\text{O N ha}^{-1} \text{ d}^{-1}\]

Figure 4. Quality control. Paired time series from the same set of samples but different gases are shown in which vial leakage has been identified by visual inspection (red point). A) CO₂ concentration over time. B) N₂O concentration over time.
Discussion

The static chamber-based approach described here is an efficient method for measurement of GHG flux from soil systems. The relative simplicity of its components makes it especially well suited to conditions or systems in which more infrastructure-intensive methods are infeasible. In order to generate high quality data, however, the static chamber approach must be carried out with strict attention to experimental design.

One notable consideration that must be taken into account is the spatial variability of soil gas fluxes, which can result in high variability among replicate chamber-based measurements. In designing experiments, therefore, it is important to include enough replicates to provide adequate power for statistical analysis. Tradeoffs may exist between the number of treatments which can be studied while maintaining sufficient replication, and a minimum of four replicates per treatment is a general guideline.

If measured fluxes will be used to estimate daily emissions, diurnal variations in air temperature, soil temperature, and gas emissions must be taken into account. If research goals require measurements to be obtained in mid-morning when temperatures reflect daily averages, the restricted window for sampling may affect the number of chambers that can feasibly be monitored. An additional consideration to be evaluated is the impact that inclusion or exclusion of plant roots and above ground biomass will have on gas fluxes. Chamber placement relative to plant tissue will impact the interpretation of flux data, particularly in the case of CO$_2$ where not only microbial respiration but also root and shoot respiration and photosynthesis must be appropriately balanced. For additional discussion of these factors, see Parkin and Venterea.

As noted previously, many variations on this methodology exist, including chamber design and sampling volume. One such variation is in the method employed to transfer samples between the syringe and collection vial. The technique described here first flushes the collection vial with sample before filling the vial to positive pressure. A more commonly used technique is the transfer of samples from syringes to vials that have been pre-evacuated using a vacuum pump, and the use of non-evacuated vials without flushing has also been reported. Another significant point where a range of approaches exists is in data analysis and the selection of the flux model most appropriate to the system under study. In addition to the linear regression method described here, non-linear models may also be employed, particularly when longer deployment times are used. These models include the algorithm developed by Hutchinson and Mosier and derivations thereof, the quadratic procedure described by Wagner, and the non-steady-state diffusive flux estimator described by Livingston et al. For a thorough discussion of non-linear flux models, refer to Parkin et al. and Venterea.

Methods similar to the static chamber approach include the use of flow-through measurement systems with Fourier transfer infrared (FTIR) spectrometry as an alternate to syringe sampling and gas chromatography, as well as automation of chamber closure and sampling through various means. Automated systems enable more frequent measurements with reduced personnel, but also require additional infrastructure investments. Grace et al. provide an extensive summary of options and tradeoffs in automated chamber-based N$_2$O measurement.

Characterization of greenhouse gas flux from both managed and natural systems is important to inform process-based models, understand the impacts of management practices and inform mitigation strategies, and to support global accounting and climate change modeling. Thus while individual studies are informative at the local scale, much additional value is derived through contributing to, and drawing from, a global body of knowledge on gas exchange between the landscape and the atmosphere. It is key, therefore, that data be collected and reported in a way that ensures longevity and interoperability with the broader knowledge base. This includes following best practices to ensure data quality, as well as collection of ancillary measures and comprehensive reporting of metadata to allow extension of findings beyond discrete studies. Excellent guidelines for data reporting are available from the GRACEnet project and the GRA.

Disclosures

The authors have nothing to disclose.
References


Materials List for:
Measurement of Greenhouse Gas Flux from Agricultural Soils Using Static Chambers

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URL: http://www.jove.com/video/52110
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<th>Materials</th>
<th>Company</th>
<th>Catalog Number</th>
<th>Comments</th>
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<td>Labco Limited</td>
<td>719W</td>
<td>Collection vials</td>
</tr>
<tr>
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<td>N/A</td>
<td>Chamber anchor and lid - bottom cut out of anchor, holes for septum and vent tubing bored in lid</td>
</tr>
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<td>Wheaton</td>
<td>W224100-173</td>
<td>Chamber septa for syringe sampling - insert into hole bored in lid top</td>
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<td>Sigma-Aldrich</td>
<td>Z685623</td>
<td>Chamber vent tubing - insert in hole bored in lid side, flush with exterior, approximately 25 cm coiled in lid interior (a 1 ml syringe tip may be used as an attachment mechanism)</td>
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<td>Adhesive foam rubber tape or HDPE O-ring</td>
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<td>Chamber sealing mechanism - fastened to underside of lid rim</td>
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<td>409818</td>
<td>Insulating and reflective coating - affix to exterior of chamber lid</td>
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<td>Staples / McMaster</td>
<td>831610 (Staples) / 1863A21 (McMaster)</td>
<td>Lid attachment mechanism - for clamping lid to anchor during sampling</td>
</tr>
<tr>
<td>Gas chromatography equipment fitted with electron capture detector for nitrous oxide, infrared gas analyzer or thermal conductivity detector for carbon dioxide, flame ionization detector for methane</td>
<td>Various</td>
<td>N/A</td>
<td>For sample analysis</td>
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