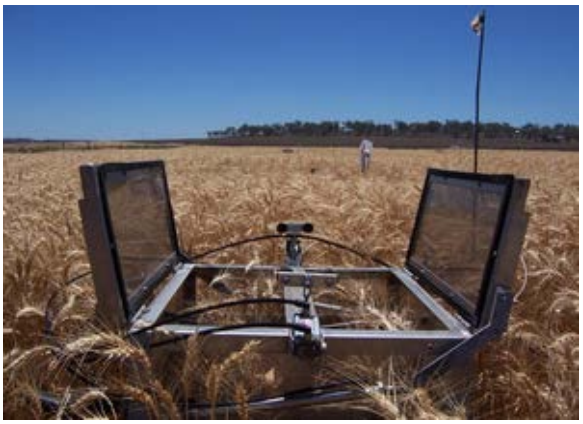
Nitrous Oxide Chamber Methodology Guidelines



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GLOBAL RESEARCH ALLIANCE ON AGRICULTURAL GREENHOUSE GASES

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Version 1.1

Editors

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Nitrous Oxide Chamber Methodology Guidelines
Cecile de Klein & Mark Harvey (eds)

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List of Corrected Errata

P102: in subsection 6.1.2.2, the sentence immediately after Eq. 2 should read "where k = D/Hd...." instead of "where = D/Hd....". (Corrected 28/7/2015)

EXECUTIVE SUMMARY

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Background

For the last 30 years, static (or 'non-steady state') chambers have been most commonly used method for measuring N_2O fluxes from agricultural soils. The main advantages of this technique are that it is relatively inexpensive, versatile in the field, and the technology is very easy to adopt. Consequently, much of the knowledge and understanding of N_2O emissions that underpins the estimation of national emission inventories from agricultural soils and efficacies of potential mitigation practices is based on N_2O chamber measurements. More than 95% of the thousands of published N_2O emission studies used chamber methodologies – in particular, non-flow-through, non-steady-state (NSS) chambers.

Non-steady-state chambers rely on the accumulation of N_2O within an open-bottomed chamber placed on the soil surface. Headspace samples are usually taken once on each sampling day and analysed in the laboratory using gas chromatography to estimate the daily N_2O flux of each chamber. Flux measurements are then made from a given number of chambers over a given time period, and a given sampling frequency, to determine spatially and temporally integrated N_2O emissions. The key aspects of chamber methodologies all have the potential to bias results or bias third-party interpretation of those results, and therefore limit inter-study comparisons and assessment of the reliability and uncertainty associated with the results. The international science community increasingly recognises the need for standardised guidelines on the use of chambers – and associated data reporting – for measuring N_2O emissions from agricultural soils.

In 2011/12, the New Zealand Government, in support of the objectives of the Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases, funded an international collaboration to progress the development of guidelines and recommendations. At an initial workshop in New Zealand in May 2011, leading experts from Alliance member countries reviewed the current state of understanding of N_2O chamber methodologies, and developed the outlines for this guideline document. Since then, researchers from around the world have been working together to write the chapters for the different steps in producing and reporting N_2O flux data from the use of chambers.

This document details the current state of knowledge of N_2O chamber methodologies and provides guidelines and recommendations for their use. In developing the guidelines, each chapter covers one of the key aspects – including design, deployment, air sample collection, storage and sample analysis, data analysis and data reporting – with additional chapters on automated systems and Health and Safety. Each chapter outlines: i) agreed minimum standards, ii) site or system specific requirements and iii) evolving standards. The minimum requirements and evolving standards are summarised here, and at the start of each chapter. For site- or system-specific requirements please refer to the individual chapters.

The guidelines define minimum requirements, but are not highly prescriptive. They aim to provide practitioners with information on best practice and factors that need to be considered in design and operation of N_2O flux measurement programmes. Areas where there is no current consensus are described as ‘evolving issues’. A major discussion point that emerged was the difficulty of having to balance limited resources between carefully measuring individual fluxes, versus increasing the number of chambers and/or sampling occasions to account for spatial and temporal variability. Understanding the size of the uncertainties of each step of the chamber measurement approach, and their impact on relative uncertainty of estimated cumulative emissions and emission factors, will be of critical importance for balancing (limited) resources to achieve the best possible (most accurate) results.

Minimum requirements

Chamber design

Chapter 2 of these guidelines discusses chamber design recommendations with a focus on static chambers. Design requirements summarised below seek to maximise flux detectability and minimise any measurement artefacts (chamber biases) associated with poor design.

Design feature	Minimum requirements
Materials	Inert to N_2O, such as stainless steel, aluminum, PVC, acrylic
Area	Recommendation is for chamber area: perimeter ratio to be ≥10 cm.
Height	Chamber height (cm) to deployment time (h), ratio should be ≥40 cm h^{-1}.
Base depth	Ratio of insertion depth: deployment time of ≥12 cm h^{-1}. Height above soil surface should be as close to the soil surface as practical (<5 cm).
Gas tight seal	A water trough or rubber/closed-cell foam gasket. Gaskets should have low internal cross-sectional area, and be compressible. Appropriate fasteners are required with rubber gaskets.
Sampling port	Inert rubber septa or syringe taps.
Vent while placing chamber on base	Opening a vent or sampling port while placing the chamber is recommended.
Vent during deployment	*No consensus on whether vents should be used or not – evolving issue.* However, if used, vents should be located close to the soil surface, or be designed to withstand wind. Appropriate vent dimensions are dependent on expected wind speeds during deployment, and should be adjusted accordingly (see references in text). Chambers and their vents should be bench-tested to ensure no Venturi effect occurs.
Insulation	Use reflective foil, foam, polystyrene. Test effectiveness by comparing surface soil temperatures inside and outside the chambers.

Deployment protocol

Chapter 3 of these guidelines discusses the deployment protocol. In addition to chamber *design*, good practice in *deployment* is also important to achieve the acquisition of best quality data for emission estimation. In addition to the individual chamber deployment, there are recommendations for designing plot experiments for group deployment, replication and for accompanying environmental measurements that should be made.

Deployment issue	Minimum requirements
Site disturbance	Chamber bases to be inserted at least 24 h prior to the first sampling – preferably longer, if logistics allow it. Avoid disturbance of the soil around the chambers. Relocate chambers when soil water content within the chamber differs from surroundings.
Chamber deployment	For chambers with a maximum height of 20 cm, use a deployment period ≤ 30 – 40 min.
Chamber sequence and grouping	Ensure that measurements are sampled per block, rather than per treatment, to ensure each block is sampled in the shortest possible period. Whenever possible, vary the block sampling sequence between sampling days, to avoid potential bias.
Number of samples per flux measurement	Three headspace samples per flux measurement, especially at times when high emissions are expected[1].
First air sample (T0)	Take T_0 sample immediately after chamber placement on the base[2].
Ancillary measurements	Measure soil texture, bulk density, pH, oranic C and total N content at least once for each campaign. Measure average soil and air temperature and total rainfall hourly or daily. Measure soil water content on each sampling day.
Time of day	Studies suggest that between 10 am and 12 noon reflects daily average, but whenever possible, researchers need to determine the diurnal pattern of N_2O emissions to assess time of day that best represents the average daily flux for their study.

[1]When high spatial variability of fluxes requires an increase of chamber replication and resources are limited, two or three headspace samples may be taken. However, researchers must quantify any bias that may be introduced by assuming a linear increase in headspace N_2O concentration.

[2]When ambient air samples are used as an estimate of T_0, researchers need to establish that the N_2O concentration in T_0 samples taken from within the chambers is not significantly different from ambient air.

Duration of experiment	Continue measurements until there is no significant difference in N_2O emissions, or driving variables of N_2O emissions between treated and control plots. For emission factor measurements for inventory, measurements should ideally cover 12 months.
Frequency of sampling	When N_2O peak fluxes are expected, sample at least twice per week, PLUS sample one to two days prior and one to two days after any event likely to induce peak emissions. During periods of low N_2O flux, sample at least once a week. When fluxes have returned to background levels, the sampling interval can be further increased.
Size and number of chambers	Chambers should cover an area as large as practical, while providing information at the smallest scale for which it is needed (see also Chapter 2).
Placement of chambers	Assess if spatial gradient in fluxes exists, divide area into relatively homogenous areas and stratify sampling accordingly. In absence of spatial structure, place chambers randomly.
Treatment replication	A minimum of three replicate plots is needed, preferably more.

Sample collection, storage and analysis requirements

Chapter 4 of these guidelines outlines best practice for analytical lab determination of N_2O gas samples, calibration requirements for optimal accuracy and how to assess adequate analytical precision.

Sampling issue	Minimum requirements
Sample collection and storage	Clean, non-reactive material that can be sealed; container evacuation recommended.
Sample analysis by GC	Commercially-made GC system; flow control and automated sample injection recommended.
Reference gases **GC system calibration**	Confidence in the N_2O concentration of all standards. Similar ranges of standards and samples, and many 'ambient checks' recommended.
Processing GC data	Determine repeatability standard deviation of standards and air samples.
Sample analysis and N_2O fluxes	Determine repeatability standard deviations for the air samples and associated N_2O flux.

Automated chambers

Chapter 5 of these guidelines discusses the underlying requirements for successful deployment of automated chambers. While a relatively new technology, a variety of design solutions have been successfully deployed to date.

Deployment issue	Minimum requirements
Materials	Stainless steel frames and transparent acrylic panels.
Gas leaks	Non-reactive adhesives to form a tight seal and prevent leaks.
Pressure changes	2-3 mm vent (this is normally of the same design as the gas sample port) to minimise any pressure artefacts.
Representative gas sample	A single sampling port at the top of the chamber is sufficient in volumes < 50 litres. Larger chambers are prone to within chamber gradients.
Site disturbance	Two chamber bases per replicate plot, to be inserted at least 24 hours prior to the first sampling. Switch chamber position between bases every week to minimise site disturbance artifacts.
Chamber air temperature	Air temperature sensor within at least one chamber (in a block design) to trigger opening at a pre-defined temperature (<<50 deg C)). In high temperature environments, tinted panels or insulation may be required on chambers.
Precipitation and rainfall	Rain gauge with threshold set to open all chambers.
Chamber sequence	Ensure that measurements are sampled per block, rather than per treatment, to avoid bias through sampling order.
Ancillary measurements	Profile soil texture, bulk density, pH, organic C and total N content at least once per season. Sample soil for mineral N (0-10 cm) every month (deeper increments preferred). Weather station nearby. Sensors for continuous logging of surface soil and air temperature, and soil moisture.
Power, weather proofing and security	Trailer or shed with access to mains power within 100 m, including UPS, or remote power source: e.g., solar, with backup generator.
Calibration and carrier gases	Spare gas cyclinders and calibration gases, so continuous operation is not interupted.
Chamber or sampling line leakage or blockage	An infrared CO_2 analyser (e.g. LI-COR®) will provide high temporal-resolution data which can provide a rapid graphical assessment of leaks. Ensure ascarite (H_2O absorber) is regularly changed. Regular visual inspection of chambers and sampling lines.
Data quality and continuity	Regular visual assessment of graphical outputs will reduce the risk of poor or lost data. Regular computational analysis of data. Computer back-ups.

Data analysis

Chapter 6 of these guidelines discusses data analysis considerations. Guidance is provided to allow selection of the most appropriate flux calculation method, how to best interpolate non-continuous measurements to obtain best estimates of emissions and emission factors.

Analysis topic	Minimum requirements
Selection and use of a flux calculation method	Method should be matched to the number of headspace samples taken (see Table 6.2, and also Chapter 3 - Chamber Deployment).
Estimation of emissions using non-continuous flux data	Daily fluxes can be integrated, using trapezoidal integration. To improve the accuracy of cumulative emissions estimates, maximise sampling frequencies and spatial replication given available resources. Repeat experiments over multiple years, and consider using spatial or temporal gap filling procedures.
Assessment of minimum detectable flux (MDF)	Determine random measurement error associated with sampling and analysis of replicate standards of known concentration, and use the resulting error rates to determine MDF.
Statistical considerations for analysing inherently heterogeneous flux data	If treatments are replicated (at least three blocks), the variability between replicates can be assessed by calculating means of chambers in each replicate. The variability within the replicate can also be determined by assessing the chamber variability.
Estimation of emission factor (EF)	Requires the inclusion of no-N control treatment, and the subtraction of cumulative emissions in control from cumulative emissions in experimental treatment(s) receiving N addition.

Data reporting

Chapter 7 summarises the minimum requirement for reporting data, which includes experimental site details, methodology details, ancillary measurement details and analysis details.

Safety precautions

In Chapter 8, the Health and Safety (H&S) risks associated with all stages of chamber measurement – from field deployment through to laboratory and subsequent analyses – are discussed. It is important that research staff consider these prior to starting any chamber deployment and sampling. Issues are identified, along with personal protective equipment (PPE) and hazard minimisation procedures, which should be considered as a minimum when complying with institutional and national legislation.

Evolving Issues

At the time of producing these guidelines, there are a number of areas where there is lack of consensus.

Chamber design

The use of a chamber vent during the flux measurement	*There remains a lack of consensus amongst researchers, with many opting not to vent during chamber deployment due to possible Venturi effects.Further data sets pertaining specifically to N_2O fluxes are needed to resolve this issue.*
Headspace mixing	*Effects of mixing should be tested and reported on. There has been very little work done on evaluating specific requirements for best approach to mixing.*

Deployment protocol

Ancillary measurements	*N_2O data sets are generated for many purposes, including the parameterisation and validation of models. Researchers should consult with modellers on any additional model input parameters that need to be measured, and at what frequency.*
Accounting for diurnal variability in fluxes	*Measure diurnal pattern of driving variables to assess if daily N_2O flux should be corrected.*
Sampling frequency	*Determination of sampling strategies that optimise temporal and spatial coverage of soil N_2O fluxes.*

Data analysis

Flux calculation method	*Criteria for site-specific selection of best non-linear scheme need to be developed.*
Estimation of emissions using non-continuous flux data	*Use of automated chamber systems (see chapter 5) can help minimise temporal uncertainties, but better estimates of spatial variability require a very large number of chambers, or the use of non-chamber (e.g. micro-meteorological) methods.*
Assessment of minimum detectable flux (MDF)	*Different flux calculation schemes can differ in their MDF, therefore the choice of flux calculation scheme can change MDF (see evolving issues for section 6.1).*
Statistical considerations for analysing inherently heterogeneous flux data	*To assist with comparison of heterogeneous chamber datasets, record the spatial coverage of observations (chamber area times the number of chambers, relative to the plot size: i.e., the plot area covered by the chambers). Advanced techniques are being developed to improve description of non-normal and spatially heterogeneous data-sets and use this to select the best method for mean estimation.*
Estimation of emission factor (EF)	*Non-linearity of N_2O response to N addition needs to be assessed in different systems (i.e., EFs may vary, depending on rate of N input).*

1 INTRODUCTION

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This document provides internationally agreed reference guidelines for measuring N_2O emissions using chamber methodologies, so as to inform the production of quality N_2O flux measurement data and improve inter-comparability between international studies.

The measurement of nitrous oxide (N_2O) fluxes from agricultural soil surfaces can broadly be categorised into two main measurement techniques: chamber and micrometeorological methodologies (Denmead 2008). These guidelines deal exclusively with the former. For the last 30 years, static chamber methodologies have been most commonly used to measure N_2O fluxes from agricultural soil surfaces. More than 95% of the thousands of published N_2O emission studies used chamber methodologies – in particular, non-flow-through, non-steady-state (NSS) chambers (Rochette 2011).

This technique has several advantages: it is relatively inexpensive, versatile in the field and the technology is conceptually easy to deploy. Consequently, N_2O chamber measurements have informed the bulk of our knowledge and understanding of N_2O emissions, which in turn underpin the estimation of national emission inventories from agricultural soils, and the modelling of those processes.

A recent review of chamber methodology N_2O emissions studies from around the world highlighted large variations in chamber design, deployment and data analysis (Rochette & Ericksen-Hamel 2008). When they evaluated each of these aspects, Rochette and Ericksen-Hamel (2008), concluded that more than half of the 356 studies were of “poor” or “very poor” quality when judged by their ‘robustness’ criteria. It is important to note here that Rochette and Eriksen-Hamel (2008) could only judge the quality of the studies by the information provided in the published papers. It is therefore possible that studies were categorised as “poor” or “very poor”, even though they might have met the ‘robustness’ criteria, simply because it was not reported in the paper.

Nevertheless, the large variability in chamber design and use, and the associated potential errors in the estimations, could have major implications for the reliability and inter-comparability of N_2O emission data reported in the literature.

There is an urgent need to reduce the environmental impacts of agriculture, and to curtail climate change due to CO_2 – and non-CO_2 – greenhouse gas emissions. Therefore, international inventory reporting obligations – and efforts to reduce GHG emissions – require accurate, reliable N_2O flux measurements. Due to the large variation in current approaches to N_2O chamber design, deployment and data analysis, – and associated uncertainty around the quality of the results – there is a widely-recognised need for standardised approaches and guidelines.

This document summarises the key considerations and recommendations – as agreed by an international panel of experts – on using chamber methodologies for measuring N_2O emissions from agricultural soils. Each chapter discusses a factor critical to the accurate determination of N_2O fluxes: chamber design, deployment protocols, gas-sample collection and analysis, automated chamber systems and data analysis and flux calculation methodologies.

The recommendations are summarised as either 'minimum requirements', 'site specific considerations', or 'evolving issues' (those issues for which no clear consensus currently exists). Adopting these guidelines and considerations will improve the quality of reported measurements and subsequent calculated emission factors. They will improve the ability to compare results between studies, and help guide scientific peer-review when assessing the quality of submitted chamber measurements. In these guidelines, we also provide recommendations on minimum requirements for data reporting, and discuss health and safety considerations when using chamber methodologies.

Key discussion points

A major discussion point that emerged during the preparations of these Guidelines was the difficulty of having to balance limited resources and the scarcity of experimental evidence to make informed decisions and judgements. On the one hand, a larger number of headspace samples per chamber is likely to provide the best possible individual flux measurement. On the other hand, N_2O emissions are notoriously variable (both spatially and temporally) and a large number of replicate chambers as well as a high sampling frequency are required to minimise the variability of the cumulative N_2O emission estimates.

Underlying this debate is the question of whether one can assume that the N_2O concentration inside the chamber increases linearly, or how large the potential bias in the flux estimate is by assuming linearity. Diffusion theory suggests that the accumulation of N_2O inside the chamber immediately suppresses the vertical gradient in N_2O concentration as soon as the chamber is put in place: i.e., the N_2O increase is non-linear. However, some researchers have shown that the N_2O increase during chamber deployment is very close to linearity. If that is the case, then the number of headspace samples per flux measurement can be lower, and the available resources

can be used to increase chamber replication and/or sampling frequency. However, there is insufficient experimental evidence to clearly determine the trade-offs between carefully measuring individual flux measurements versus increasing the number of chambers and/or sampling occasions to account for spatial and temporal variability.

Understanding the size of the uncertainties of each step of the chamber measurement approach – from the number of headspace samples in each individual flux measurement, to chamber replication, sampling frequency and flux calculation and integration method – and their relative impact on calculating cumulative emissions and emission factors, will be of critical importance for balancing limited resources to achieve the best possible (most accurate) results.

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2 CHAMBER DESIGN

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Summary table

Table 2.1: Summary of considerations when designing non-steady state chambers

Design feature	Design objective	Minimum requirements	Site specific issues	Evolving issues
Materials	To prevent gas exchange through chamber.	Inert to N_2O, such as stainless steel, aluminum, PVC, acrylic.	Robust frames required to withstand grazing.	
Area	Minimise error due to poor sealing. Maximise area sampled.	A chamber area-perimeter ratio of ≥10 cm is recommended (equates to a cylindrical chamber of ≥40 cm diameter).	Adaptation needed if rocks or roots are present, or if required by research objectives.	
Height	Maximise flux detection and minimise perturbation of environmental variables.	Chamber height (cm) to deployment time (h), ratio should be ≥40 cm h^{-1}.	Chamber height should accommodate crop height.	
Base depth	Prevent below ground lateral gas transport, shading and ponding of water.	Ratio of insertion depth to deployment time of ≥12 cm h^{-1}. Height above soil surface should be as close to the soil surface as practical (<5 cm).		
Gas tight seal	Prevent gas leaking between chamber and base.	A water trough or rubber/closed cell foam gasket. Gaskets should have low internal cross sectional area, and be compressible. Appropriate fasteners are required with rubber gaskets.		

Design feature	Design objective	Minimum requirements	Site specific issues	Evolving issues
Sampling port	For extracting sample.	Inert rubber septa or syringe taps.		
Vent while placing chamber on base	To prevent pressure disturbance while placing the chamber on the base.	Opening a vent or sampling port *while placing the chamber* is recommended.		
Vent during deployment[a]	To prevent pressure gradients between the interior and exterior of the chambers during flux measurement and gas sampling.	If used, vents should be located close to the soil surface, or be designed to withstand wind. Appropriate vent dimensions (diameter and length) are dependent on expected wind speeds during deployment, and should be adjusted accordingly (see references in text). Chambers and their vents should be bench-tested to ensure no Venturi effect occurs.		There remains a lack of consensus amongst researchers, with many opting not to vent during chamber deployment due to possible Venturi effects. Further data sets pertaining specifically to N_2O fluxes are needed to resolve this issue.
Insulation	Prevent temperature gradients between the interior and exterior of the chambers.	Use reflective foil, foam or polystyrene. Test effectiveness by comparing surface soil temperatures inside and outside the chambers.		
Headspace mixing	Well-mixed headspace to ensure representative sample is taken.	If active headspace mixing (e.g., fans) is required, it should not affect the diffusive flux.	Crop type and chamber height.	Effects of mixing should be tested and reported on. There has been very little work done on evaluating specific requirements for best approach to mixing.

[a]Note: Note there remains a lack of consensus amongst researchers with many opting not to vent during chamber deployment due to possible Venturi effects and further data sets pertaining specifically to N2O fluxes are needed to resolve this issue.

2.1 Introduction

Chamber designs may employ flow through, non-steady state or steady state chambers (Denmead 1979), or non-flow through, non-steady state chambers (Rochette & Eriksen-Hamel 2008). However, the literature on N_2O emissions is dominated by the use of non-flow through, non-steady state chamber methodologies (Bouwman *et al*. 2002), often referred to as 'static chambers'.

Since chambers are invasive, nuances in chamber design can affect the accuracy of N_2O flux determination (Parkin *et al*. 2012) and the subsequent upscaling of results. This is because chambers can change the vertical diffusion of N_2O in the soil, the soil energy balance, and degree of turbulence above the soil (Rochette 2011).

This chapter provides recommendations on minimum requirements, and discusses the key principles for chamber designs to minimise the impact of the measurement technique on the natural soil and atmospheric processes. It provides guidance and recommendations on materials, dimensions, venting, seals, insulation, sampling port, plant effects and headspace mixing. Some examples of recommended design are shown in Appendix 1.

2.2 Materials and components

Above all, chamber materials should not react with any gases from the soil system. Neither should they emit any contaminants into the atmosphere above the soil surface, nor the soil itself, once positioned. Recommended materials so far include stainless steel, aluminium, polyvinyl chloride (PVC), polycarbonate, polyethylene, or polymethyl methacrylate (Plexiglas®, acrylic sheet) (Parkin & Venterea 2010). Other factors, such as the presence or absence of plants, may also influence the choice of material, as discussed below.

Any other components used in chamber construction, such as seals, tubing, septa and venting, should also be inert. The chamber system should also be robust. If used in grazed pasture studies, chamber materials must be rigid, so as to prevent chamber flex and to withstand treading and chewing by grazing animals. Heavy mesh cages may be needed to stop cattle damaging chambers.

In the past, chambers have been as simple as 'push-in' covers pressed into the soil. Nowadays, the use of such chambers is strongly discouraged, since they disturb the soil gas profile immediately prior to the flux determination. This disturbance happens when the chamber wall disturbs aggregates, roots and biota in the soil profile as it is inserted, affecting gas containment, transport and production processes, and the piston flow of air that results from chamber placement (Hutchinson & Livingston 2001, Matthias *et al*. 1980).

Instead, chambers should consist of a paired 'base-chamber' design, where the chamber is placed and sealed onto a base – sometimes also referred to as an 'anchor' or soil collar (Parkin & Venterea 2010) – previously inserted into the soil. Depending on the application, insertion time before measurements begin can vary from hours for a

bare coarse-textured soil to weeks when insertion results in roots damage. Other considerations around chamber design, as discussed below, are venting, sample ports, effective sealing, soil temperature monitoring and insulation.

2.3 Dimensions

Good chamber design must consider certain critical dimensions, such as the internal chamber height above the soil surface, the chamber area (cm^2) and the length (cm) of the chamber perimeter. These last two factors are used to calculate the chamber area-perimeter ratio, which Rochette and Eriksen-Hamel (2008) recommended should be ≥ 10 cm (e.g. a cylindrical chamber of 40 cm diameter), based on work by Healy *et al*. (1996). This is because the relative error associated with any poor chamber seal decreases as the diameter of a chamber increases (see below). Chamber area will depend on where the apparatus is deployed: larger chambers can of course be placed on relatively flat, clear terrain, but forest ecosystems might require chambers of smaller area. In either case, the chamber will ideally be as large as feasibly possible in order to capture spatial variation. Chambers covering an area up to 2 m^2 have been used, but most common models have an area smaller than 0.5 m^2.

A chamber's geometry is important when dealing with spatial variability problems at small scales (Rochette & Hutchinson 2005). For example, in a row crop, nitrogen fertiliser banding and soil compaction in the inter-rows often produce a flux gradient perpendicular to the plant rows. If a research objective is to describe that gradient, long, narrow rectangular chambers are most appropriate. If a description of the inter-row gradient in flux is unnecessary, then chambers covering the whole inter-row are most efficient. In grazed pasture systems, chambers are often circular, so as to enclose the generally circular area of animal urine patches. Smaller chambers may also be required in particular studies exploring the spatial variability of fluxes: e.g., the effect of animal hoof compaction versus non-compacted soil surrounding the hoof print.

Height is another critical feature of chamber design. As chamber height increases, the impact on environmental variables such as humidity, or the N_2O diffusion gradient within the soil, is reduced. However, the minimum detectable flux increases (Hutchinson & Livingston 2002; Rochette & Eriksen-Hamel 2008). Conversely, if the chamber height is decreased, the minimum detectable flux is reduced, but at the expense of greater perturbation of the system (temperature, humidity and gas concentration). The significance of these perturbations – and their dependence on chamber height – is intrinsically linked to chamber deployment time, so Rochette and Eriksen-Hamel (2008) devised a ratio of chamber height (cm) to deployment time (h), recommending that well-designed and deployed chambers have a ratio of ≥ 40 cm h^{-1}.

Of course, experimental objectives might also determine chamber heights. If the aim is to capture the role of a tall plant, such as wheat, this will dictate the chamber height. However, the user needs to be aware that detectable fluxes will be lower, so the closure period may need to be extended. Also, uniform N_2O concentrations may not be present within the chamber at time of sampling (see below). One option is to use chamber extensions. These are sections used to extend the height of the chamber as

the plant grows, but care needs to be taken with the seal between chamber extensions, and mixing of the headspace, particularly around extensive crop canopies.

Dimensions of the base obviously need to match the chamber, so as to achieve a gas-tight seal. However, other dimensions, and the design of the chamber base, also need to be considered. Atypical soil moisture conditions within the chamber must be avoided, because water retention in the base after rain or irrigation could change soil aeration, soil temperature and microbial processes. While the chamber is left open, the base must not be so high that chamber plots are partially or fully shaded, since this could change soil temperature and lead to unintended effects on soil moisture and microbial processes. Thus, the wall of the base exposed above the soil surface needs to have a very low profile. Parkin and Venterea (2010) recommend base walls no higher than 5 cm: however, chamber bases can be designed to be almost flush with the soil surface (Parkin & Venterea 2010).

Another critical dimension is how far the chamber base is inserted into the soil. Failure to push it deeply enough into the soil can allow N_2O to leak, or ambient air to contaminate the chamber headspace via lateral diffusion of gases through the soil, as a consequence of the vertical N_2O concentration gradient being disrupted (Rochette & Eriksen-Hamel 2008). To prevent artefacts, the base walls need to be inserted to at least the depth where N_2O concentrations are being perturbed by feedback effects of the chamber, so as to prevent lateral diffusion of N_2O beneath the wall. (Healy *et al*. 1996; Hutchinson & Livingston 2001).

Hutchinson and Livingston (2001) modelled the relationship between deployment time, air-filled porosity (0.1, 0.3 and 0.5 $cm^3\ cm^{-3}$) and the base insertion depths required to reduce lateral diffusion by either 1% or 5% of the steady state N_2O flux. Their results indicated that a 5 cm insertion depth was more than sufficient in soil with low effective diffusivity (soil air-filled porosity ≤ 0.1 $cm^3\ cm^{-3}$). However, it was only adequate for brief deployment periods (20 to 30 min) at a soil porosity of 0.3 $cm^3\ cm^{-3}$, and inadequate at higher values of soil air-filled porosity (0.5 $cm^3\ cm^{-3}$). Their data indicate that, for deployment times of 30 min, insertion depth should be 10 cm at a soil air-filled porosity ≤ 0.3 $cm^3\ cm^{-3}$, increasing to 20 cm if air-filled porosity is as high as 0.5 $cm^3\ cm^{-3}$.

Rochette and Eriksen-Hamel (2008) concluded in their review study that a ratio of insertion depth to deployment time of ≥ 12 cm h^{-1} was very good. A prior knowledge of maximum soil air-filled porosities at the site of chamber deployment can help reduce errors, and the data of Hutchinson and Livingston (2001) should be consulted for guidance.

For discussion of soil installation recommendations, see Chapter 3.

2.4 Venting

After going to the trouble of establishing gas-tight seals, and inserting bases to appropriate depths, it seems incongruous to then employ openings, or vents, in the chamber covers. However, published evidence clearly supports the use of vent tubes

on non-steady state chambers (Bain *et al*. 2005; Davidson *et al*. 2002; Hutchinson & Livingston 2001; Hutchinson & Mosier 1981; Xu *et al*. 2006). Vents prevent pressure gradients between the interior and exterior of the chamber from influencing gas exchange. Pressure gradients can occur when the chamber is placed on its base, and during the sampling of the chamber headspace (Christiansen *et al*. 2011). However, opening a vent during chamber placement stops this happening. Inadequate insulation may cause pressure differentials to develop in unvented chambers, as a result of cooling or warming of the chamber air (Davidson *et al*. 2002). Naturally occurring pressure gradients may occur outside the chamber as a result of wind-driven turbulence (Rochette 2011). If the turbulence-driven changes in barometric pressure are reduced due to a chamber's placement over the soil surface, N_2O emissions will be reduced inside the chamber (Hutchinson & Mosier 1981).

Higher N_2O fluxes have been reported when vents have been used in chambers (Hutchinson & Mosier 1981). In another study, the use of vents increased measured N_2O fluxes five-fold in a well-aerated soil, but reduced them in less permeable soils, suggesting that vents might create greater problems than they solve (Conen and Smith, 1998). Well-designed vents transmit barometric pressure fluctuations while minimising leaks (i.e., N_2O diffusion out of the chamber via the vent tube) and contamination (i.e., the intake of external ambient air into the chamber during gas sampling, or temperature induced pressure changes inside the chamber).

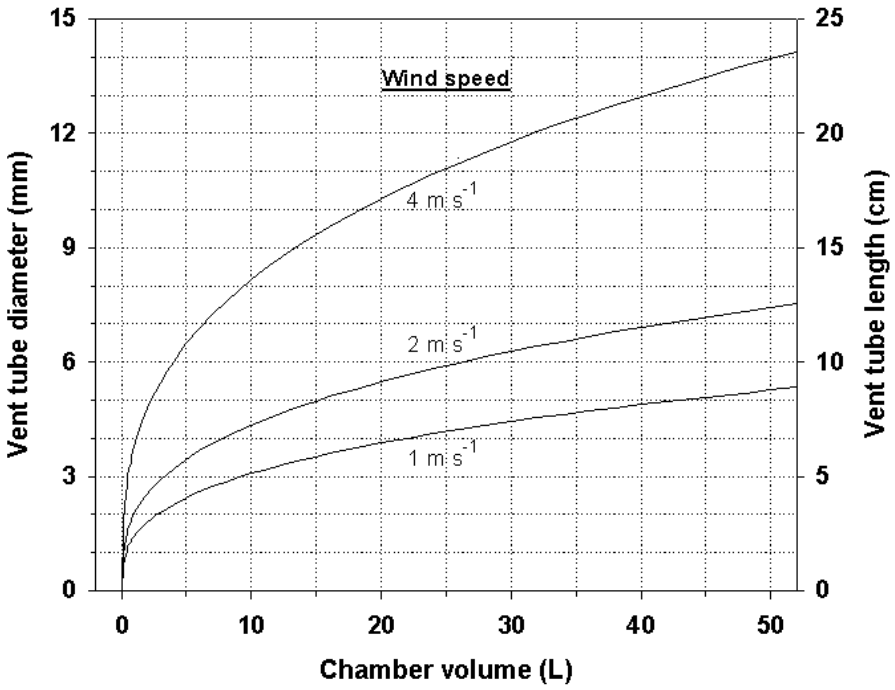
A vent tube is constructed from inert tubing, and secured through the chamber with an appropriate gas-tight bulkhead fitting. Criteria for optimal vent design, given by Hutchinson and Mosier (1981), stated that: (i) the tube diameter (D) must be small enough to minimise diffusive losses, but large enough to permit air – moving in response to pressure changes – to flow down the tube with pressure loss no greater than 0.1 µ bar, and (ii) the vent tube length (L) must be not less than that which gives an internal volume five times greater than the volume of enclosed air displaced by the largest anticipated pressure wave.

The equations provided by Hutchinson and Mosier (Hutchinson & Mosier 1981), which relate wind speed, D and L, must be used to calculate the optimum vent tube dimensions for the conditions of the chamber study, such that the loss of accumulating N_2O by diffusion never exceeds 1% (Hutchinson & Mosier 1981). A further practical guide to selecting vent tube length and diameter as a function of chamber volume and wind speed, based on Hutchinson and Mosier (1981), is provided by Parkin and Venterea (2010), Figure 2.1.

However, the use of vents has also been shown to induce a further source of error, due to wind flowing over the vent outlet and creating a Venturi effect, which depressurises the chamber (Bain *et al*. 2005, Conen & Smith 1998, Suleau *et al*. 2009, Xu *et al*. 2006). Hutchinson and Livingston (2001) noted that the explanation for the results obtained by Conen and Smith (1998) was inconsistent. Because large pressure differences also occurred between vented and non-vented chamber types when wind speed and soil air permeability were smallest, they wondered if temperature-driven expansion had caused an effect.

Davidson *et al*. (2002) noted that there were possible artefacts in both directions for the vented and non-vented chambers used by Conen and Smith (1998), making it difficult to know which chamber yielded the 'true' flux. Davidson *et al*. (2002) did, however, measure an average internal chamber headspace pressure difference of -0.2 kPa on a moderately windy day, when a vented chamber was over a soil surface, but summarised their findings by stating that errors due to chamber pressure artefacts can be minimised – or almost eliminated – by appropriately sized vents. Hutchinson and Livingston (2001) stated that the weight of evidence is in favour of vents, and that, so long as vents are adequately designed, adverse effects are minimised. Potential Venturi effects can be further minimised by correctly sizing the vent, mounting it as close as possible to ground level to minimise wind speed and by pointing the vent outlet downwind – maybe even shielding it in strong winds. Bain *et al*. (2005) confirmed the Venturi effect described by Conen and Smith (1998) using flow through, non-steady state chambers (5 L PVC with vertical vent tube (0.19 cm diameter, 3.56 cm long)) attached to either impermeable bases, or a PVC base inserted 2 – 4 cm into the soil. For the chambers on the impermeable bases, the fan-controlled wind conditions in the field resulted in negative chamber pressures with a ca. 1 Pa drop in pressure per 1 m s^{-1} increase in horizontal wind speed at chamber height. When this was repeated on natural soil, there were no pressure changes inside the chambers. All experimental variables were similar, and a negative pressure should have been induced. Bain *et al*. (2005) concluded that mass flow of gas through the soil was occurring, and compensating for the chamber pressure gradient.

Figure 2.1: Optimum vent tube diameter and length for selected wind speeds and enclosure volumes as described by Hutchinson & Mosier (1981), extracted from Parkin and Venterea (2010)



Advection of a soil gas with this flow will increase the estimated gas flux. This same effect was observed by Xu *et al*. (2006), who recorded no negative pressures inside a chamber placed on a base sitting on soil, but found higher CO_2 fluxes in windy conditions. They subsequently found negative chamber pressures when the chamber was connected to an impermeable base. The lack of negative pressures with the chamber placed on the soil was due to mass flow of soil air into the chamber headspace. Such wind effects on mass flow will vary with soil moisture and porosity, and associated error will also depend on gas concentration (Xu *et al*. 2006).

The Venturi effect has been overcome by improved vent design that virtually eliminates the occurrence of artificially induced pressures changes (-15 to 8 kPa) under windy conditions of up to 6.5 m s^{-1}(Xu *et al*. 2006). With wind speeds up 4 m s^{-1} at chamber height, Xu *et al*. (2006) showed that flux overestimates of up to 19% occurred in CO_2 flux calculations when the soil CO_2 flux ranged from 0.5 to 2.5µmol^{-2} s^{-1}. A study by Suleau *et al*. (2009) examining soil respiration fluxes confirmed previous findings: that the Venturi effect can occur *in situ*, and they offered an alternative design to overcome the effect.

Suleau *et al*. (2009) found that locating vents (of their own design) 0.05 m above the soil surface reduced previous overestimates of flux (≤ 300%) in strong winds. To ascertain whether a particular chamber design invokes pressure gradients (Venturi effect) at wind speeds expected under field conditions, the chamber must be tested by sealing the base to an impermeable surface while wind speeds and internal chamber pressures are monitored (Bain *et al*. 2005; Suleau *et al*. 2009; Xu *et al*. 2006). The use of vents remains an evolving issue, despite these recent findings. If vents are used, they should be tested to show no Venturi effects under deployment conditions.

2.5 Seals

An essential element of a multiple component chamber is the gas-tight seal placed between the two components. This is commonly achieved by placing a rubber gasket between the chamber and its base (Parkin & Venterea 2010) or using a built-in trough, attached to the base, that holds water and acts as a seal between the two components (Christiansen *et al*. 2011; Hutchinson & Livingston 2001). Specifications for the material(s) required to form the perfect seal between components have never been clearly defined. Obviously, the aim is to prevent N_2O leaking out of the chamber and external air into the chamber. Modelling by Hutchinson and Livingstone (2001) clearly showed that gasket material must have a very low internal cross-sectional area available for diffusion (i.e., a very low diffusivity), and must be pliable enough to form a good seal when compressed. Hutchinson and Livingston's (2001) simulation used a 0.25 cm wide by 0.25 cm high foam gasket which, at simulated porosities of 0.001 to 0.03, provided gas losses equal to 0.055 and 2.3% of the total mass flux, respectively.

In all cases, the modelled gas loss was greater through the simulated leaking gasket than through the vent – sized for a wind speed of 4 m s^{-1} (Hutchinson and Mosier 1981) – which was only 0.038% of the total mass flux. The study also highlighted the importance of eliminating gaps between the abutting seal and the component. Such

gaps only needed to be 1.2 to 53 µm respectively, to achieve the same loss of gas flux as achieved through diffusion through the gasket. This stresses the importance of using precision-machined chamber components. Some form of fastener is often used to compress the chamber against the base's gasket, ensuring a tight seal. A seal's effectiveness can be tested by placing concentrations of a reference gas inside a chamber sealed to an impermeable surface, and measuring the rate of N_2O concentration change over time. Ideally, there should be no changes in gas concentrations over typical deployment periods. Water seals have their shortcomings: they are only useful on flat ground; they can dry out and can become dirty with algal growth. A supply of clean water is required at each sampling, and care must be taken not to spill water into the chamber, where it could affect the potential for N_2O production. Otherwise, water seals are very effective and a generally preferred option for flat sites.

2.6 Insulation and temperature control

Soil temperature can affect N_2O production and reduction rates, the solubility of N_2O in soil water, and the diffusion rate of N_2O. Likewise, if the chamber is not vented, any temperature decreases or increases in the chamber will lead to negative or positive pressure effects respectively (Rochette and Hutchinson 2005). Parkin and Venterea (2010) calculated that, if not corrected for, significant temperature changes (> $5^oC\ h^{-1}$) will produce errors in calculated fluxes. Xu *et al*. (2006) note that, according to the Ideal Gas Law, a 1^oC change in chamber temperature could result in a 333Pa change in chamber pressure. Chamber placement can also impact on soil temperature and on biological processes that produce or consume N_2O. It may also indirectly affect N_2O production. For example, a 1^oC increase in soil temperature may increase soil respiration by 10% at 10^oC, in the absence of other limiting factors (Lloyd & Taylor 1994).

Thus, any increase in soil temperature may enhance CO_2 emissions, which in turn could alter the soil's oxygen status and indirectly affect N_2O production mechanisms. Any increase in the concentration of other gases resulting from chamber placement can affect N_2O concentrations (Rochette & Hutchinson, 2005). For example, Parkin and Venterea (2010) demonstrate how an increase in water vapour concentration – a consequence of increasing temperature elevating humidity in the headspace – could decrease N_2O concentrations by 3% (this is known as the water vapour dilution effect). This in itself may not cause an underestimation of the N_2O flux, since the final effect will depend on other factors, such as linearity of the flux over time.

The aim of insulating the chamber, then, is to preserve and maintain the initial air and soil temperature present at the time of chamber placement. This may be achieved by covering the chamber, outer walls and dorsal surface with either a reflective foil or an insulating material, or preferably a combination of both. Regardless of method, it must be proven satisfactory by comparing measured air and soil temperatures inside and outside the chamber during typical deployment periods and conditions.

Where plants are present, chamber studies may employ transparent covers, but these create significant problems with maintaining internal chamber temperatures. Temperature control mechanisms can be implemented (Don Herman, UC Berkeley, pers. comm.), but in their absence, flux measurement periods need to be kept short to minimise temperature effects, and temperatures need to be monitored so corrections can be made if required.

2.7 Sampling port

A sampling port is required to remove a gas sample from the chamber. It should be inert and gas tight, except when samples are taken. Butyl rubber septa and syringe taps sealed to the chamber are often used. Septa materials must be inert, and changed at regular intervals to prevent leaks. The use of syringe taps may create 'dead' air spaces that remain unexposed to the increasing gas concentration in the headspace. Care must be taken to purge these during the gas sampling process. Sampling ports can be connected to a tube that samples air at several locations within the headspace to minimise problems associated with concentration gradients.

2.8 Allowing for plant effects

Plants can have significant effects on N_2O fluxes (Chang *et al*. 1998; Jørgensen *et al*. 2012; Pihlatie *et al*. 2005; Reddy *et al*. 1989; Yu & Chen 2009). In some cases, a chamber cover may influence the rate of such effects on the N_2O flux. For example, placing an opaque chamber cover on the soil surface, over the top of plants, will block incoming radiation, which in turn will lead to stomatal closure (Hopkins & Hüner 2009). This in turn can reduce any subsequent N_2O flux as a result of dissolved N_2O being transported in the transpiration stream. The magnitude of any artefact will depend on soil moisture, humidity and the dissolved N_2O concentration, while the significance will depend on other components of the total N_2O flux derived from the soil surface.

Smart and Bloom (2001) found that wheat leaves could emit N_2O during assimilation of nitrogen. The rate increased 10-fold when the N source was switched from ammonium to nitrate, and they found that N_2O production was associated with photo-assimilation of nitrite in the chloroplast. This process is recognised in many plant species (Yu & Chen 2009). Blocking sunlight with opaque chamber materials, therefore, may reduce this N_2O flux source. However, the relative significance of plant-derived N_2O production is not well understood. Again, the magnitude of any reduced N_2O flux will depend on plant species, the amount of biomass (leaf surface area) enclosed by the chamber, inorganic N forms in the soil and their amounts. The significance of any such effect will depend on the relative N_2O flux from the soil itself.

Few studies have examined the potential artefact(s) – or their magnitude – that may result from the use of opaque materials during chamber N_2O flux measurements. If plants are enclosed in transparent chambers, there is clearly a conflict between the need to insulate the chamber to limit air temperature changes, and a need to maintain solar radiation for plant function. Thus, researchers need to be aware of these issues when designing experiments specifically to look at plant effects on N_2O fluxes.

2.9 Headspace mixing

Manual gas sampling and mixing of headspace air in non-flow through, non-steady state chambers can potentially affect soil surface gas exchange, and lead to a bias in results (Christiansen *et al*. 2011; Liu & Si 2009; Rochette & Eriksen-Hamel 2008; Rochette & Hutchinson 2005). Manual gas sampling of chambers is the commonest method of sampling N_2O concentrations, with potential artefacts of manual sampling minimised by selecting appropriate sample volumes for example < 1% of headspace volume.

Modelling has shown that soil gas fluxes can be underestimated if the air inside the chamber is not constantly mixed during the enclosure period (Liu & Si 2009). Fans have been used to mix headspace air in closed chamber headspaces, to overcome possible bias from vertical gas concentration gradients. Jørgensen *et al*. (2012) mixed the headspace of their chambers immediately prior to measurement, to eliminate vertical concentration gradients in chambers containing plants 60-110 cm high. However, few studies have specifically examined the effects of fan-mixing in chambers, especially on N_2O fluxes. Rochette and Hutchinson (2005) showed that, for a 60 L square chamber without fans, the CO_2 flux was highly variable, but when a single fan was used, CO_2 flux determinations were generally higher than unmixed fluxes. However, the results were inconsistent over time, and no benefit was obtained from multiple fans (two or four).

Using sand beds, Christiansen *et al*. (2011) set up five reference methane (CH_4) fluxes (60 – 2000 µg m^{-2} h^{-1}), and studied the effects of manual sampling with syringes and fans on headspace air mixing and subsequent flux determinations, using a 68 L unvented chamber. In non-mixed chambers (no fans) syringe sampling altered CH_4 concentrations inside the chamber, leading to a 36% underestimate of the measured reference fluxes. Comparisons of reference and measured CH_4 flux estimates improved when horizontally positioned fans (68 m^3 h^{-1}) were used to mix headspace air. The fan speed did not induce mass flow of gas from the sand beds.

Christiansen *et al*. (2011) concluded that further research was required to fully understand the combined effects of chamber dimensions and mixing rates on estimated flux rates. It is likely that headspace mixing is more important in tall chambers enclosing a larger amount of biomass (such as a mature cereal crop). In theory, the perfect mixing system should align headspace mixing intensity with pre-deployment conditions (Rochette & Hutchinson 2005). This is not a simple task to achieve, but it suggests that placement of non-mixed chambers in an exposed windy environment – and of strongly-mixed chambers in calm locations (i.e., under plant canopy) – would result in the greatest flux measurement biases. At present, however, the literature is insufficient to make more specific recommendations on the use of fans.

Another technique to allow for, and to overcome, the occurrence of vertical gas concentration gradients is to use a gas sampling manifold inside the headspace. Parkin and Venterea (2010) used a simple manifold built into the chamber cover to draw headspace air from four quadrants during sampling, in order to minimise any effect of gas concentration gradients in the headspace.

2.10 Summary

Initially, researchers must consider both the objectives of the experimental programme the chambers will be used in, and the soil characteristics at the intended site(s). This – along with the principles outlined above, and further research to fine-tune them – will produce a chamber design of optimal dimensions. Before deployment, the chosen chamber design should be 'bench-tested' on an impermeable surface to ensure that materials are inert, that there are no leaks or Venturi effects at anticipated deployment wind speeds and that temperature perturbations have been minimised. Plants inside chambers create unique challenges: if the aim is to maintain plant function during the enclosure period, chamber design needs to be carefully considered. Finally, in certain deployments, significant vertical gradients may develop within the chamber and further studies are needed to assess the best way of alleviating these prior to, or during, sampling.

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3 DEPLOYMENT PROTOCOL

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Table 3.1: Summary of considerations for optimising chamber deployment

Overarching issue	Deployment issue	Objective	Minimum requirements	Site or situation specific issues	Evolving issues
Individual chamber deployment	Site disturbance.	Minimise effect of site disturbance on flux estimate.	Chamber bases to be inserted at least 24 hours prior to the first sampling – preferably longer – if logistics allow it. Avoid disturbance of the soil around the chambers. Relocate chambers when soil water content within the chamber differs from surroundings.	On clay soil under dry conditions, the seal between the soil and the chamber base may be compromised as the soil shrinks away from the edges. In such circumstances, researchers should carefully 'tamp' the soil on the outer edge of the base, to fill the gap and improve the seal.	
	Chamber deployment.	To minimise changes to the physical environment, and the risk of leaks.	For chambers with a maximum height of 20 cm, use a deployment period of 30 – 40 minutes.	If higher chambers are required due to crop height, extend deployment period.	

Overarching issue	Deployment issue	Objective	Minimum requirements	Site or situation specific issues	Evolving issues
	Chamber sequence and grouping.	To avoid bias due to sampling order or pattern.	Ensure that measurements are sampled per block, rather than per treatment, to ensure each block is sampled in the shortest possible period. Whenever possible, vary the sequence of sampling the blocks between sampling days, to avoid potential bias.		
	Number of headspace samples per flux measurement.	Ensure best possible estimate of hourly flux.	Three samples per flux measurement, especially at times when high emissions are expected[3].	When high spatial variability of fluxes requires an increase of chamber replication and resources are limited, an alternative approach may be considered – see text[3].	

[3]when only two or three headspace samples are taken, researchers must quantify any bias that may be introduced, by assuming a linear increase in headspace N2O concentration (see text for further details).

Overarching issue	Deployment issue	Objective	Minimum requirements	Site or situation specific issues	Evolving issues
	First air sample (T_0).	Ensure adequate measurement or estimate of T_0 in each chamber.	Take T_0 sample immediately after chamber placement on the base.	When ambient air samples are used as an estimate of T_0, researchers need to establish that the N_2O concentration in T_0 samples taken from within the chambers is not significantly different from ambient air.	
	Ancillary measurement.	To help explain findings, to develop functional relationships and/or determine proxy measurements.	Measure soil texture, bulk density, pH, organic C and total N content at least once for each campaign. Measure average soil and air temperature and total rainfall hourly or daily. Measure soil water content on each sampling day.	Soil NH_4^+ and NO_3^- content should ideally be measured on each sampling day, except when the flux sampling frequency is high (every few days) and changes in soil mineral are not likely to happen that rapidly. Then frequency can be reduced to times when changes are expected.	N_2O data sets are generated for many purposes, including the parameterisation and validation of models. Researchers should consult with modellers on any additional model input parameters that need to be measured, and at what frequency.
Cumulative emissions	Time of day.	To ensure that the time at which the flux measurements are taken represents the average daily flux.	Studies suggest that between 10 am and 12 noon reflects daily average, but whenever possible, researchers need to determine the diurnal pattern of N_2O emissions to assess time of day that best represents the average daily flux for their study.		Measure diurnal pattern of driving variables to assess if daily N_2O flux should be corrected.

Overarching issue	Deployment issue	Objective	Minimum requirements	Site or situation specific issues	Evolving issues
Temporal variability	Duration of experiment.	To capture the entire 'treatment-induced' emission envelope.	Continue measurements until there is no significant difference in N_2O emissions, or driving variables of N_2O emissions between treated and control plots. For emission factor measurements for inventory, measurements should ideally cover 12 months.	When measurements cannot be made (e.g. due to flooding or snow), estimate missing values based on established relationships between soil and environmental variables and N_2O flux (e.g. using models).	
	Sampling frequency.	To minimise any biases when using the trapezoidal rule to estimate of cumulative fluxes after N applications. To reduce random error associated with sampling at times of rainfall.	When N_2O peak fluxes are expected, sample at least twice per week PLUS sample 1-2 days prior and 1-2 after an event that is likely to induce peak emissions. During periods of low N_2O flux, sample at least once a week. When fluxes have returned to background levels, the sampling interval can be further increased.	When the spatial variability of fluxes is high and resources are limited, the sampling frequency may be reduced in favour of an increase in chamber replication to minimise the uncertainty in flux estimates.	Determination of sampling strategies that optimise temporal and spatial coverage of soil N_2O fluxes.
Cumulative emissions	Size and number of chambers.	To capture spatial variability of fluxes.	Chambers should cover the largest area practical, while providing information at the smallest scale for which it is needed (see also Chapter 2).	When spatial variability within a plot is expected to be high, multiple chambers within the same plot should be used.	

Overarching issue	Deployment issue	Objective	Minimum requirements	Site or situation specific issues	Evolving issues
	Chamber placement.	To provide the best representation of variability with the least number of chambers.	Assess if spatial gradient in fluxes exists, then divide area into relatively homogenous areas and stratify sampling accordingly. In the absence of spatial structure, place chambers randomly.	In cropping and grazed systems, stratify sampling into distinct statistical populations (e.g. row and inter-row or urine and non-urine patch areas).	
Spatial variability	Treatment replication.	To estimate mean emission at required level of accuracy and probability.	A minimum of three replicate plots is needed – preferably more.	Replication depends on the level of expected variability, and the likelihood of finding a significant difference between treatments. Conduct 'power' analysis to determine adequate level of replication.	

3.1 Introduction

A deployment protocol is a suite of actions and options taken when using manual non-steady state (NSS) chambers for measuring soil N_2O emissions. When estimating absolute gas emission rate values, chambers should be deployed using a rigorous methodology to ensure that the chamber deployment does not systematically bias the flux estimate, so the gaseous emission rates can be compared with data from other studies. Similarly, a rigorous deployment strategy is also required to account for temporal and spatial variability of soil N_2O emissions.

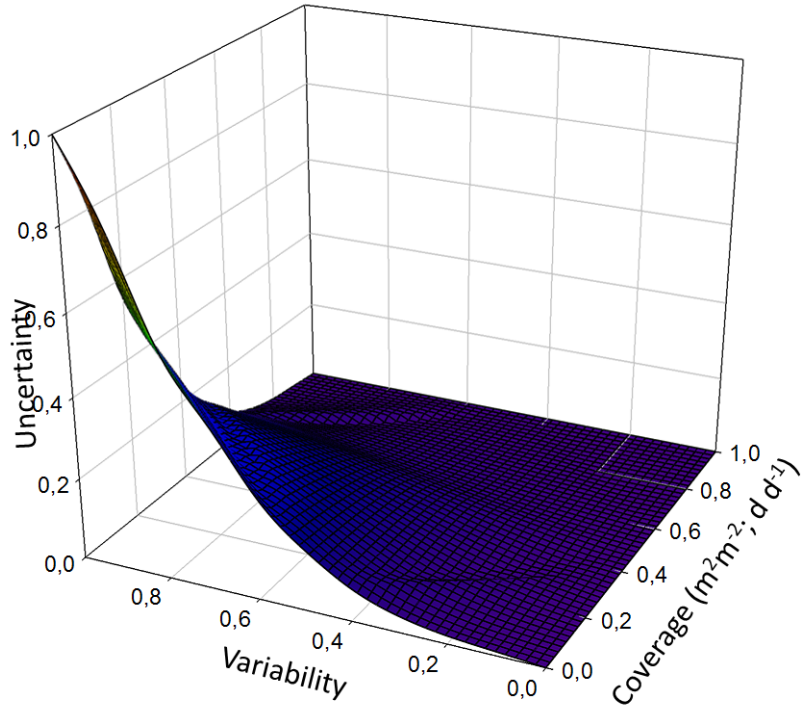
Several factors determine the suitability of a NSS chamber deployment protocol for a given situation: terrain characteristics; the presence or absence of plants; soil conditions (e.g., texture, compaction); objectives of the study; chamber geometry; other material resources and staffing. Although some procedural aspects are common to many situations, there is no unique recommendation for all field studies.

In this chapter, we grouped the recommendations in three categories, according to whether they impact on individual chamber deployments, or on temporal or spatial integration of the fluxes. As these recommendations all aim at reducing the uncertainty of emission estimates, we begin this chapter with a brief overview of the various sources of uncertainty to consider when balancing limited resources, so as to improve the overall accuracy and precision of flux estimates. Table 3.1 provides an overview summary of considerations and recommendations, with further discussion and detail provided in the sections below.

3.2 Sources of uncertainty

There are a several potential sources of uncertainty when using flux chambers to estimate representative (i) hourly fluxes for individual chambers, (ii) daily fluxes for individual chambers, (iii) seasonal/annual fluxes for individual chambers, and (iv) integrated fluxes for a plot or given area of land (Table 3.2). Levy *et al*. (2011) concluded that the selection of flux calculation method was the single largest source of uncertainly in hourly flux estimates from individual chambers. However, uncertainty associated with temporal or spatial variability of fluxes is potentially larger than uncertainty associated with hourly flux estimates from individual chambers. While temporal variability can be addressed by automatic chambers, or increased resources to sample chambers more frequently, spatial variability requires additional consideration. Figure 3.1 shows this in a conceptual form.

Figure 3.1: Conceptual representation of the impact of variability (spatial or temporal) and coverage (spatial or temporal) on the uncertainty of the soil cumulative N_2O emission estimates. Maximum values were attributed the value of "1"



At the larger scale, coefficients of variation for N_2O fluxes in California field plots have been shown to vary from 160% to 500% (Foloronso & Rolston 1984). In a cut-only sward, Velthof *et al*. (1996) reported values from 53 to 273% and estimated that between seven and 30 chambers (20-cm diameter) were required to obtain a mean within 50% of the true flux, while 375 to 1240 chambers would be needed to increase precision to within 10%.

When developing a soil N_2O measurement protocol using chambers, all sources of uncertainty should be considered, to ensure a focus on improving any aspect of the protocol that might limit the accuracy of the emission estimate. For example, carefully measuring individual fluxes, using a limited number of chambers, may be ideal over a small area with homogeneous soil properties. A simpler individual chamber protocol, however, could be used when large spatial variability requires a greater number of chambers.

Table 3.2: Overview of sources of uncertainty associated with hourly, daily or cumulative flux estimates for individual chambers, and spatially integrated flux estimates for a plot or field

N_2O flux estimate	Source of uncertainty
Hourly flux **(individual chamber)**	Equipment issues: • accuracy and range of GC standards • GC precision Choice of flux calculation model, number of headspace samples taken, and accounting for soil N_2O 'storage' (see chapter 6). Accuracy of time of headspace sampling. Total height of combined chamber plus base above soil surface. Air temperature and pressure at time of sampling vs time of analysis. Dilution of N_2O concentration, due to water vapour concentration inside the chamber.
Daily flux **(individual chamber)**	Temporal variability within day. Time of day that best reflects daily average flux.
Seasonal/annual flux **(individual chamber)**	Temporal variability (i.e., sampling frequency) within measurement period. Sampling coverage (number of sampling dates and timing). Duration of measurement period. Interpolation method.
Spatially integrated flux **(group of chambers)**	Spatial variability. Size and placement of chambers. Treatment replication. Number of chambers per treatment, or per plot. Chamber sequence and grouping.

3.3 Individual chamber deployment

3.3.1 Chamber installation and site disturbance

Installation of chambers or chamber bases causes soil disturbance, which may impact on gas emissions (Matthias *et al*. 1978; Norman *et al*. 1992). Bases should be installed long enough before measurement to allow for conditions to approximate ambient. On

bare soil, this might take as little as one hour on coarse-textured soils, while a few days may be needed on clays (Rochette, personal communication).

Installation of bases in vegetated areas often damages roots, so weeks – perhaps months – are required to allow root regrowth. This will avoid any potential impact of root death, which will disrupt C and N cycling with potential effects on N_2O production in the soil profile. If root death caused by base insertion is likely, chamber bases need to be deployed well before flux measurements to allow roots to grow back (Rochette & Hutchinson 2005). Otherwise, shallower wall insertions may be needed, but only if other criteria for good design and deployment are used (see Chapter 2). Among annual crops, bases should be installed shortly after seeding, to allow roots to grow into the inner area.

Soil water content can impact on chamber performance in several ways. Researchers walking around the chambers, especially in very wet conditions, can compact the soil. This can disturb the conditions to such an extent that N_2O production and vertical transport are modified. Walking boards reduce this, but sampling NSS chambers in saturated soil often causes so much site deterioration that collars must be relocated. Bases may also affect lateral surface water flow, and they should be relocated when soil water content differs from surroundings (Rochette & Bertrand 2008). Finally, under very dry conditions, clay soils may shrink away from the edge of the chamber base. In such circumstances, researchers should carefully loosen and tamp down the soil at the outer edge of the base prior to measurement, to fill the gap and improve the seal between the soil and the base.

3.3.2 Chamber deployment duration

Theoretically, the longer the deployment duration, the more robust the flux estimate, because it integrates the emission over a longer period. However, problems associated with changes to the chamber physical environment, and the risk of leaks, increase with deployment time. Therefore, keep NSS chamber deployments short. Based on the sensitivity of modern gas chromatographs, changes in headspace N_2O concentration that cannot be measured in 0.2 m-high chambers after 30 min usually represent flux levels (< 3 μg N m^{-2} h^{-1}) of little interest for most situations. However, where higher chambers are required – over growing crops, for instance – duration may be increased accordingly. The chamber deployment duration also depends on the number of headspace samples to be taken during the enclosure period, based on the choice of flux calculation method (discussed in detail in Chapter 6), the number of simultaneously deployed chambers and the number of field operators.

3.3.3 Sequence and grouping of chamber measurements

Grouping and sequence of chamber measurements vary depending on deployment duration, experimental design and human resources. The number of chambers that can be handled by one operator increases with deployment duration, but decreases with the number of headspace samples and distance between bases. Chamber size and height, or stacking requirement (tall crops), may also impact on the number of chambers an operator can handle. The time interval between sampling two chambers varies, depending on their location, but it is usually ≥ 60 seconds. Where an operator

samples one chamber every minute, the four air samples (0, 8, 16, 24 min) of a series of eight chambers will be completed in a total of 31 minutes.

In the case of a measurement design with repeated treatments, groups of chambers handled together should represent entire repetitions of treatments. Because flux measurement often takes longer when there are many chambers to be sampled, it is important to sample different treatments within a replicate block in as short a period as possible. This avoids temperature-induced biases, and enables statistical comparisons of fluxes. However, the sampling sequence should vary between sampling dates, to avoid any potential bias from always sampling in a particular order.

3.3.4 Headspace air sampling

When deploying chambers for measuring N_2O emissions, it is important to determine the requisite number of headspace samples to provide the least biased flux estimate. Clearly, the more headspace samples taken, the better the characterisation of the accumulation of N_2O and thus, the less biased each individual flux estimate will be.

Rochette (2011) proposed that four or more air samples should be taken during deployment, to adequately assess the quality of the calculated flux (detection of outliers and technical problems during handling and analysis of samples), and to account for the increase in non-linear rates of gas concentration with deployment time. In this chapter, we reinforce this recommendation, but also acknowledge that a less intensive chamber headspace sampling may be acceptable for certain situations. Any consideration around reducing headspace sampling intensity should be based on minimising the overall uncertainty of the flux estimate. For example, when flux spatial variability is exceptionally high, it may be preferable to deploy a greater number of less-intensively sampled chambers (two or three samples) to improve plot-level flux estimates, even if this comes at the cost of increased uncertainty in individual chamber estimates (see Section on Spatial Variability).

However, if the priority is to generate a representative flux – through the sampling of multiple chambers per plot and assumption of a linear increase in headspace N_2O concentration, rather than multiple sampling from the headspace of fewer chambers – it is essential to qualify any potential bias introduced by only taking two or three headspace samples. This can be done by taking a random subset of chambers on each sampling occasion, and conducting four or more headspace samples during the course of the two- or three-point sampling strategy (e.g. as in Cardenas *et al*. 2010). Each dataset of four or more headspace samples should be statistically analysed to determine (non-) linearity. At the end of the experimental period, researchers should summarise this information, provide a percentage of cases when linearity was observed, then cite this alongside their calculated flux. This will provide an indication of the bias – hence confidence – in the results that may have been introduced by assuming linearity in the flux calculations.

3.3.4.1 *First air sample (T0)*

At the time of deployment, the chamber is sealed onto the base for the duration of the measurement. The impact of chamber placement on the soil/headspace environment

(as well as the associated effects of soil-surface gas production/transfer) increases with time, so it is best to limit deployment duration to a minimum (≤ 30 min; Rochette & Bertrand, 2008). However, it is difficult to stop chamber placement affecting the flux, and non-linear rates of headspace gas concentration are often observed. Estimation of unbiased flux rates therefore requires determining a dC/dt representative of pre-deployment conditions: i.e., as early as possible after deployment. For this reason, special attention should be given to the first – or T_0 – air sample.

In a well-mixed atmospheric environment over a N_2O-emitting soil surface, there is little horizontal gradient in N_2O concentration at the plot scale (100 m^2), and a few ambient air samples taken at mid-chamber height can be used in place of individual T_0 samples in each chamber. However, several precautions need to be taken: since the vertical concentration profile above the soil is influenced by the flux rate, ambient air samples can only be used as T_0 estimates for chambers placed in the plot where samples were taken. Even with this precaution, a bias can be introduced if flux differs between chambers located in the same plot. Assuming that the T_0 concentrations are proportional to the flux value, using a unique ambient air concentration for a group of chambers will produce an underestimate of lower fluxes, and an overestimate of higher fluxes. This will increase variation within that group of chambers.

Second, the permanently-inserted bases need to be low, because their presence restricts lateral air flow above the surface, and usually results in near-surface N_2O concentrations greater than those in an open environment. This impact increases with increasing flux rate, base height and wind speed. For these reasons, it is recommended to take a T_0 sample immediately after chamber placement on the base, in order to avoid systematic flux estimation bias. Opting for ambient air samples in place of individual-chamber T_0 should only be selected when low bases are used, and after adequate testing indicates a low variability in flux rates among chambers that share common ambient air samples, or no significant difference in the N_2O concentration between individual-chamber T_0 and ambient air samples.

3.3.5 Ancillary measurements

Production, reduction and transport of N_2O in soils depend on the availability of C and N substrates, on gas diffusivity and on redox potential. To understand and predict N_2O net production processes and emission rates, therefore, these controlling parameters should be monitored during soil N_2O flux studies. Some ancillary measurements will be required at different frequency to others. Soil bulk density, pH, organic C and total N content are usually required to be measured once, or following an expected significant change, such as cultivation. Average soil and air temperature, and rainfall should be measured daily or hourly basis, and soil water-filled pore space at daily or weekly intervals. Soil mineral N measurements are needed as often as resources allow, especially during the first 30 days after fertiliser, manure or urine application. If the results are being used for model development or verification, more detailed and more frequent measurements may be required. These requirements are discussed in Chapter 7 (How to report your experimental data).

3.4 Cumulative emissions at the plot/field scales

Soil N_2O emissions are extremely variable in time and space. Clayton *et al*. (1994) calculated coefficients of variation of 42 to 97% (n=18) and 59 to 183% (n=6) from 0.13 and 0.49-m^2 chambers randomly spaced >50 m apart on ungrazed and grazed grasslands, respectively. At a sample spacing of 7.1 m (grid), Ambus and Christensen (1994) calculated an average coefficient of variation of 95% using 0.008-m^2 chambers on un-grazed grass, and 50% using the same-sized chambers, but placed one to five metres apart. Even at smaller spacing, spatial variability can be large: Ambus and Christensen (1994) found coefficients of variation between chambers placed 0.11 m apart of up to 55%.

Integration of cumulative soil N_2O flux values over time at field scale may be best achieved using micrometeorological measurement methods (Wagner-Riddle *et al*. 1996). However, these approaches require expensive instruments and highly qualified operators, and are not suited for experimental plots of typical size. For these reasons, most cumulative estimates such as the fertiliser-induced emission factors used for regional inventories of soil N_2O losses are determined using data obtained by NSS chambers (Bouwman 1996; Rochette *et al*. 2008).

Emissions estimates in time and space do not need to be integrated in studies comparing short-term (hourly) N_2O emissions from small plots, where chambers cover the entire soil area, However, most experiments include the estimate of cumulative emissions over time in plots much larger than the chambers. Because limited resources usually restrict chamber numbers and deployment frequency, the sampling strategy needs to be optimised to keep any uncertainty in flux estimates arising from incomplete temporal and spatial chamber coverage to a minimum. In this section, we analyse the factors controlling uncertainty associated with chamber-based temporal and spatial N_2O flux integration, and options to minimise this uncertainty.

3.4.1 Temporal integration

N_2O emissions from soils vary over time, according to seasonal and environmental patterns and farming practices, but are typically low throughout the year. Most emissions are observed during peaks that can last from a few hours to several weeks after soil disturbance, rainfall, spring thaw and additions of organic and mineral N (Rochette *et al.* 2000; Bouwman *et al*. 2002; Chadwick *et al*. 2011).

Manual NSS chambers are typically deployed for short durations, at a fixed time of day and at a low frequency. These characteristics, coupled with the episodic nature of soil N_2O fluxes, are major handicaps for integrating emissions through time. The uncertainty in estimates of cumulative N_2O emissions can be seen as a function of the temporal variation of the N_2O source, and of the temporal coverage of the measurement period by chambers (Fig. 1). The deployment protocol should aim to minimise the impact of these two factors on the accuracy and precision of cumulative flux estimates at the diurnal and seasonal/annual scales.

3.4.1.1 *Diurnal variations*

Daily emissions are often estimated from a single measurement made at the time of day when the flux is believed to equal its daily mean. In the absence of transient fluxes following a disturbance of soil N_2O producing processes (N application, tillage and rainfall), fluxes are mostly controlled by soil temperature (Livesley *et al*. 2008). Research has generally indicated that sampling fluxes when temperature in the plough layer is close to its daily mean is often indicative of the average daily flux (Laville *et al*. 2011). Smith and Dobbie (2001) reported that samplings at 0300, 1100 and 1900 hours yielded fluxes similar to daily values, while estimates by Parkin (2008) at 0600 and 1200 hours were 14% lower and 8 % greater, respectively than daily means. Measurements by Alves *et al*. (2012) in Scotland and Brazil suggested that in both countries, despite the contrasting climatic conditions, the times that best represented the daily mean 0900-1000 and 2100-2200.

However, using soil temperature to determine the time of N_2O flux measurement is not straightforward. Surface emissions lag behind the time of N_2O production at a given depth because of its vertical transport (Clough *et al*. 1999). Also, the time of day when fluxes at the surface equal the mean daily value varies according to the depth of production and soil gas diffusivity. Soil temperature should also be measured at the depth of maximum N_2O production – information that is variable, and usually unknown. Periodic measurements of the diurnal pattern in soil N_2O emissions during an experiment are an adequate way to determine when a single sampling time is representative of mean daily fluxes. However, such measurements require resources that few projects can afford, and temperature in the plough layer remains the most frequently used index for guiding time of flux measurement.

Most experimental designs and measurement protocols assume that diurnal emissions patterns are the same on all treatments and throughout the year. However, this may not always be the case. For example, if treatments affect the amount of crop residues at the soil surface, the time of daily minimum and maximum soil temperature at a given depth will likely differ among treatments. Similarly, placing nitrogen fertilisers at different depths can also produce different temporal patterns in surface fluxes. Corrections can be made using 'flux vs. temperature' relationships but fully accounting for these biases is difficult (Parkin and Kaspar 2006).

3.4.1.2 *Seasonal/Annual variations*

3.4.1.2.1 *Duration of the experiment*

Emissions of N_2O are influenced when soil C and N dynamics are modified by changes in soil physical properties or substrate availability. In studies intended to quantify the emissions induced by a climatic event or an agricultural practice, measurements should continue for as long as soil properties impacting on the N_2O emission are changed by the event/practice (the entire 'treatment-induced emission envelope'). If the measurements are to be used to determine of emission factors (EF) for soil N_2O losses inventories, they must ideally be taken over a year. However, where it involves estimating emission factors for a specific nitrogen source (e.g. N fertiliser application

or a urine patch) measurement duration could be shorter, as long as the full N-induced emission envelope has been captured (i.e., measurements to continue until the emissions and available soil N are no longer different from a control treatment).

There are specific challenges in measuring fluxes over long periods. Soil compaction from repeated footprints next to the sampling sites can bias flux measurements by modifying gas production and vertical transfer (see section on Chamber Installation and Site Disturbance above). Also, there are instances when soil conditions are not suited to NSS chamber use such as when soil is flooded or covered by thick snow. The resulting gaps in the coverage of annual emissions must then be estimated by other means, such as modelling (gap filling).

3.4.1.2.2 *Temporal coverage*

Chambers are deployed for short periods (< 1 h) and at relatively long intervals (from one to 14 days). Therefore, they provide direct estimates of the soil N_2O fluxes for a very small fraction of the time over which they are intended to estimate the cumulative emissions (month, season, year). Consequently, it is crucial to select an adequate number and time of sampling events when linear interpolation is used between sampling points for temporal integration of emission (Brumme & Beese 1992; Smith & Dobbie 2001). The maximum number of sampling dates during an experiment is finite, and depends mostly on available resources, number of chambers and the site characteristics (distance from the laboratory, spatial arrangement of plots). Therefore, sampling frequency can vary from daily, for simple experiments located at nearby sites, to weekly or more for those at remote locations. A fixed interval is often used during the measurement campaigns, but a better option is usually to vary the frequency based on whether emission peaks – due to such triggers as rainfall or fertiliser application – are expected or not.

N_2O emissions peaks typically last between one and 30 days. As peak duration and chamber deployment frequency decrease, the error associated with time-integrated emissions of a soil N_2O emission peak will increase (Parkin 2008). Maximum errors are observed when an emission peak occurs between two consecutive deployments, and when infrequent measurements coincide with short-lived peaks. While errors are expected for any given emission peak, they can be minimised by sampling as frequently as resources allow – not less than twice a week (Flessa *et al*. 2002) – when peaks are expected, and by measuring one to two days before, and after, events such as N application, rainfall and tillage to establish the pre-event baseline, and to describe the early response of soil N_2O emissions.

However, if pre-event flux measurements are not possible, a pre-event emission should be estimated – based on the last actual measurement – to ensure that interpolation of the pre- and post-event emissions is not overestimating cumulative emissions. A rapid turnaround of gas sample analysis allows for inspection of the results, and helps determine when the sampling frequency can be reduced.

Between high flux episodes, emissions can be integrated by linear interpolation between representative daily values, measured weekly, to capture the seasonal variations in emissions. Because the weighting given to individual measurements

increases as sampling frequency lessens, intervals greater than seven days can produce a significant bias when fluxes are fluctuating. Therefore they are only adequate when conditions are conducive to near-zero fluxes. The sampling interval should only be extended beyond seven days when emission increases are unlikely (Parkin 2008).

3.4.2 Spatial integration

Soil is not a homogeneous medium, and most ecosystems (including agronomical plots) can be viewed as a mosaic of N_2O sources of various intensities (Yanai *et al*. 2003; Matthews *et al*. 2010). Given the relatively small size of NSS chambers, obtaining accurate spatial integration of soil N_2O fluxes is therefore challenging, even for relatively small areas (100 m^2). The uncertainty in estimates of plot-size N_2O emissions can be seen as a function of spatial heterogeneity of the N_2O source, and of the coverage of plot area by chambers (Fig. 1). In the following sections, we analyse various ways to manage these two factors to minimise uncertainty.

3.4.2.1 *Heterogeneity of the N_2O source*

3.4.2.1.1 *Site selection*

Total soil N_2O fluxes at a given time and place can be seen as the sum of naturally occurring emissions (also called 'background emissions') and those induced by an imposed condition (N addition, soil tillage, crop type, etc.) (Pennock *et al*. 2006). Thus, in experiments to determine emissions from a particular practice, selecting small, uniform areas consistent with the measurements being made helps to minimise interference from spatial heterogeneity in background emissions. The location of these relatively homogeneous areas within a landscape – such as a grazed paddock or cropped field – can be determined before the experiment, using exploratory flux sampling. However, while this approach usually helps reduce uncertainty in estimates of practice effects, it does not account for interactions with other soil factors influencing N_2O dynamics across a given landscape.

3.4.2.1.2 *Practice*

Any interference between treatment effects and background spatial variability can be reduced with appropriate precautions during field or plot operations. The number of replicate measurements can sometimes be reduced by decreasing, or knowing, the spatial variability of the emissions prior to the experiment, depending on the hypothesis being tested. For example, an experiment designed to measure the effects of adding manure, animal urine, crop residues, synthetic N fertiliser etc. can be conducted in two ways: prescribed amounts of N can be manually applied to the chambers *in situ* within their sub-plot, or N can be mechanically applied to the whole sub-plot before placing the chamber. The first method usually reduces spatial variability, compared to deploying the chamber after a broadcast application (Rochette *et al*. 2008).

This approach reduces apparent spatial variability, but it does not account for the combined effects of soil heterogeneity, nor for the impact of uneven distribution of the amendments by farm machinery on soil N_2O emissions. So while it helps reduce

the impact of spatial variability on uncertainty in N_2O flux, is not recommended if the objective is to also account for the impact of typical field operations on N-induced emissions.

3.4.2.1.3 *Strategic sampling*

Chamber location should be selected so as to obtain the required information using a minimum number of measurements. The placement of chambers on a given area varies, depending on whether a spatial variation pattern in flux is present or not. Flux spatial structure could be determined by exploratory measurements prior to the monitoring period, or by assuming that flux will vary according to soil properties or landscape features. Also, conducting flux measurements prior to applying treatments provides information on pre-existing spatial patterns of emissions, which can be used as covariates in subsequent statistical analysis of the results. In the absence of any flux spatial structure, chambers should be located randomly, as is the case for many small agronomical plots on flat homogeneous land.

However, where flux gradients occur, the area should be divided into relatively homogeneous emitting sections, and measurements should be stratified accordingly (Davidson *et al*. 2002). Spatial integration of the fluxes can then be obtained by weighting chamber estimates, based on the proportion of the total area they represent. For example, on sloping terrain, separate estimates can be made for the drier eroded shoulders and for the wetter alluvial slopes (Pennock *et al*. 2006). Similarly, row crops may produce inter-row gradients in soil water and nitrogen content, which can be accounted for by an adequate sampling pattern: e.g., by placing chambers so as to include both row and inter-row areas (Cai *et al*. 2012).

In grazed pasture systems, where the majority of the N_2O emissions come from animal urine patches, stratifying the sampling into two distinct statistical populations, such as 'urine patch' and 'non-urine patch' areas, is recommended. This can be done by applying known amounts of urine N to specific areas, then measuring the emissions from both patches and the non-urine patch areas between them. Field scale emissions can then be calculated on the basis of urine patch area coverage:

$$N_t = (N_1 * P_1) + (N_2 * P_2) \quad (1)$$

Where N_t is the total N_2O emission from a grazed field, N_1 andN_2 are the N_2O emissions from the urine and non-urine patch areas, respectively, as measured using the NSS chambers, P_1 and P_2 are the proportion of the field covered by urine and non-urine patch areas, respectively. The values of P_1 and P_2 will vary, depending on the stocking rate and the urine patch area coverage. Finally, the spatial structure in gas emission pattern may change during the growing season (Rochette *et al*. 1991) and flux sampling strategies need to be tailored accordingly.

3.4.2.2 *Spatial coverage*

3.4.2.2.1 *Size of chambers and plots*

It is important to design measurements at a scale relevant to the research questions being addressed. Soil N_2O emissions can vary by more than one order of magnitude over short distances (Ambus & Christensen, 1994). In studies seeking to understand spatial variability, multiple small chambers can be used to determine its magnitude. Otherwise, increasing the area covered by chambers, or reducing the plot size, are the first obvious steps in integrating spatial variability. In summary; the plots should be as small as possible, and the chambers should cover an area as large as practical, while providing information at the smallest scale for which it is needed.

3.4.2.2.2 *Number of chambers per plot*

For a given chamber size, plot area increases with the number of chamber deployments. Deployment of NSS chambers and the associated air sampling and analysis are resource-intensive. The number of chambers per plot that can be deployed at any given time depends on available resources and the headspace air sampling intensity. Spatial coverage can, however, be extended by increasing resources, reducing the number of plots or reducing the number of air samples taken.

Inadequate spatial coverage often produces emission uncertainty levels that prevent clear interpretation, so one should first consider increasing the resources devoted to the project, and/or reducing the number of plots being monitored. The success of soil N_2O experiments often depend on planning decisions. Monitoring soil N_2O emission from large, sometimes remote, plots over several months requires considerable staffing and equipment, and researchers should ensure the project's resources are proportional to the scope of the objectives. When resources are insufficient for an adequate number of chambers, one may consider reducing the number of plots being monitored. In a typical agronomic project, this involves either reducing the number of treatments being investigated, or the number of replicates. Spatial variability is a major challenge, so any decision to reduce the number of replicates below four should not be taken lightly, lest it decrease statistical analysis power below an acceptable level.

The third option is to reduce the workload around individual chamber deployment protocol. This is best done by decreasing the number of headspace air samples taken during deployment, as the lower sampling frequency directly impacts on the sampling and analytical workloads. However, reducing the number of headspace air samples increases the uncertainty in individual chamber flux calculations (see chapter 6), and this may offset the benefits of an increased spatial coverage.

Moreover, insufficient air sampling may result in biased flux estimates that will directly result in similarly biased plot-scale N_2O emission estimates. It is therefore best to explore other ways to increase the number of chambers per plot (see above), before opting to reduce headspace air samples per deployment below four. Combinations of research objectives and terrain characteristics that demand a high number of chambers per plot may call for a decrease in air sampling intensity (see section 3.3.4).

In these circumstances, further precautions need to be taken to ensure that this modified protocol does not result in biased flux. They must also ensure that reduction in uncertainty due to greater spatial coverage is not offset by a greater uncertainty, because of less robust flux determination in individual chambers (see Chapter 6).

3.5 Conclusion

Inadequate deployment protocols for NSS chambers can result in biased or highly uncertain soil N_2O estimates that make their interpretation difficult. In this chapter, we reviewed the precautions necessary when deploying NSS chambers, so as to obtain reliable soil N_2O flux estimates at various temporal and spatial scales. We first outlined the requirements for individual chamber deployments, insisting on an adequate sampling of the chamber headspace to facilitate quality assessment, and accounting for chamber feedbacks on soil N_2O emissions.

We then discussed how individual chamber deployments could be most efficiently used to obtain temporal and spatial integrations of the emissions. In situations of high variability, this protocol may need to be adapted to allow more chambers to be deployed, so as to best estimate cumulative emissions at the plot/field scale. However, under all circumstances, precautions need to guard against the impact of individual chamber protocol changes on soil N_2O flux estimates.

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4 AIR SAMPLE COLLECTION, STORAGE AND ANALYSIS

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Table 4.1: Summary of sample collection, storage and analysis requirements

Topic	Objective	Minimum requirements	Potential issues	Evolving issues
Sample collection and storage	Contain air sample with low and known contamination or leakage.	Clean, non-reactive material that can be sealed; container evacuation recommended.	Compatibility with gas chromatography (GC) system.	
Sample analysis by GC	Accurately quantify N2O in sample.	Commercially made GC system; flow control and automated sample injection recommended.	System drift over time and variability. Address potential analytical artifacts due to CO_2 and H_2O in samples.	
Reference gases	Provide accurate standards for calibrating GC system.	Confidence in the N_2O concentration of all standards.	Error(s) in the calculated N_2O concentration in the samples.	
GC system calibration	Accurately determine N_2O concentration in sample.	Similar ranges of standards and samples, and many 'ambient checks' recommended.	System drift over time and variability.	
Processing GC data	Assess GC performance, and quantify uncertainties.	Determine repeatability standard deviation of standards and air samples.	System drift over time and variability.	
Sample analysis and N_2O fluxes	Calculate N_2O fluxes from the GC N_2O concentration data.	Determine repeatability standard deviations for the air samples and associated N_2O flux.	Insufficient ability to detect N_2O fluxes.	

4.1 Introduction

In this chapter, we describe the collection and storage of air samples, and the method of analysis to determine their N_2O concentrations by gas chromatography (GC). We share our experience by describing the methods and systems used in our laboratories, which builds on the earlier and wider experience of others. Where appropriate, we introduce the underlying principles for guidance for quantifying analytical data repeatability / reproducibility (equations (1) – (4)).

In this spirit, we also offer illustrative calculations to make a connection between N_2O sample analyses and the determination of N_2O fluxes from soils. In this chapter, the volumetric concentration units for N_2O will be μL L^{-1}. Assuming ideal gas behaviour, this is equivalent to a wet air mixing ratio of the number of N_2O molecules per million molecules of air (ppb), including the water vapour. The conversion to a mass basis, based on gas laws, is shown later by equation (5). It is recommended practice in atmospheric science for mixing ratio to be expressed as a dry air mole fraction (removing measurement variability due to water vapour content). Gas samples collected from static chambers can be dried before analysis, but if not, Appendix 1 discusses this error in more detail. For further background, readers are also directed to Global Atmosphere Watch (GAW) Report No. 185 (WMO 2009) http://www.wmo.int/pages/prog/arep/gaw/documents/WMO_TD_1478_GAW185_web.pdf, which provides comprehensive guidance on air sample analysis, including quality control and assurance.

4.2 Collection and storage of air samples

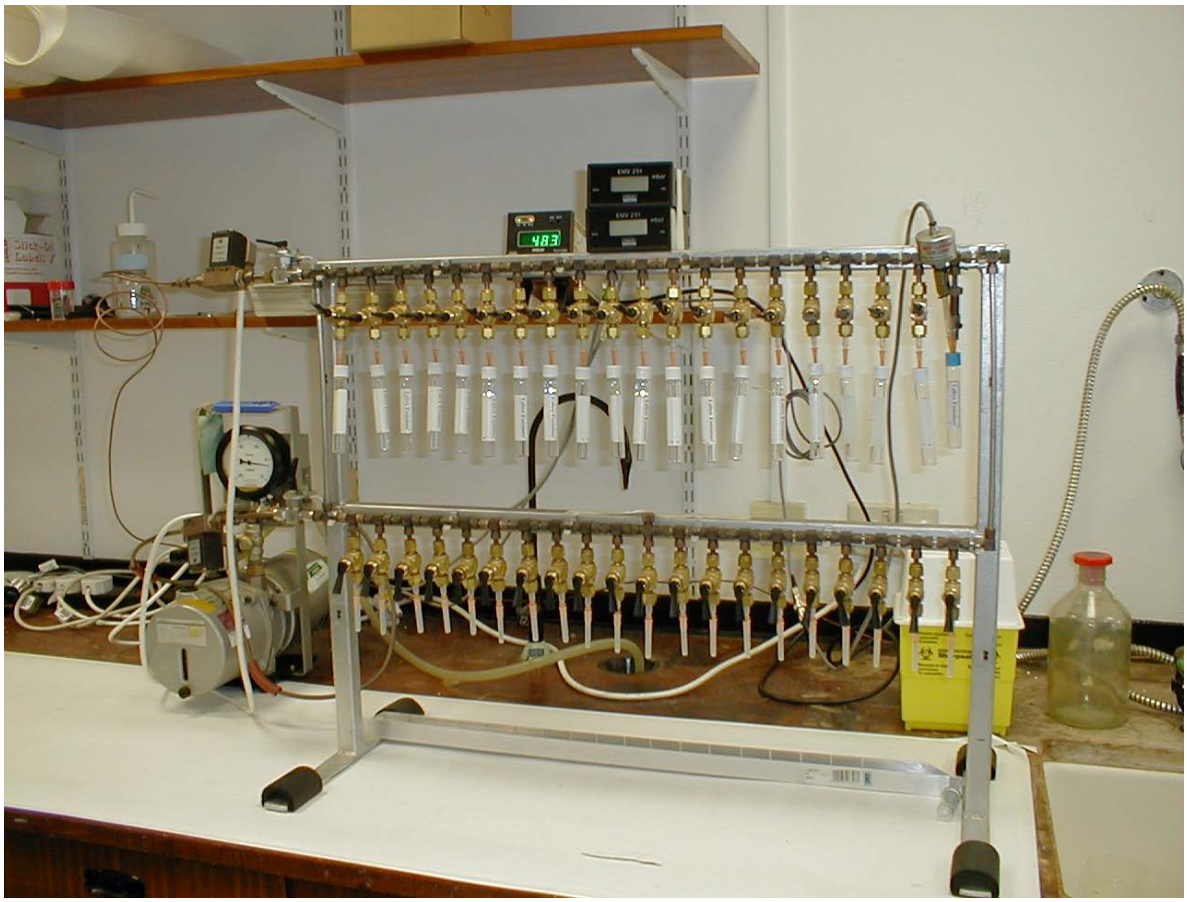
Air sample containers need to be leak-proof, clean and made of material(s) which do not react with N_2O. While good quality gas-tight syringes may be suitable for short-term storage of samples, they are an expensive option. We recommend septum-sealed containers that are evacuated (< 100 Pa) prior to sampling, which means the container must not only be able to withstand this process, but maintain the vacuum, prior to sampling. The container should remain gas-tight afterwards to prevent sample loss during storage until analysis. Rochette and Bertrand (2003) discuss issues and report the results of a comparison of polypropylene syringes and glass vials.

As an alternative to evacuation, de Klein *et al*. (2003) described an equally effective flushing procedure, in which, before sample collection, 25 mL of chamber head space air was flushed backwards and forwards through a 6 mL septum-capped glass vial container 4 times. Glass vials (e.g. Exetainer®, Labco Limited, High Wycombe, UK) are now commonly used as air sample containers, and procedures have been developed for their use. While different sizes are available, 6 and 12 mL septum-capped glass vials are most commonly deployed with air sample volumes as small as 1 mL being removed for analysis (Rasmussen *et al*.1976; Hedley *et al*. 2006). Such glass vials have screw-on plastic caps with rubber septa. Experience shows that gas tightness is achieved when the cap is screwed on 'finger tight', followed by another quarter-turn. Different septa are available.

At New Zealand's National Centre for N_2O Measurement (NZ-NCNM), 3-mm-thick, grey-coloured butyl rubber septa have been used, while at the Agri-Food and Biosciences Institute in Northern Ireland (AFBI), the septa consist of a double-wadded 'sandwich' of 3 mm-thick butyl-rubber septum and a teflon/silicone septum (also see Rochette and Bertrand 2003).

If vials are to be evacuated, a system begins with a pump (Figure 4.1). As an example, at NZ-NCNM, a dry pump is used, isolating the bearings and their hydrocarbon lubricant from the vacuum space (model XDS5, 5 for a peak pumping speed of 5 m^3 h^{-1}, Edwards, Sanborn, NY, USA). The pump is connected by tubing to a vacuum gauge and by further tubing to a manifold. Depending on the number of samples, a number of manifolds may be required, and at NZ-NCNM, three 14-port manifolds (model WMF6000, SJ4 Manufacturing Services, Inc., Cape Coral, FL, USA) have been connected in series to the pump. Each port has a two-way valve and needle (25G 5/8 needle, Becton, Dickinson and Company, Singapore, product reference 301805). The recommended needle is a 25-gauge, stainless-steel tube, 3/8 inch (10 mm) long, with 0.50 and 0.24 mm nominal outside and inside diameters, respectively.

Figure 4.1: A (12 mL) vial evacuation system, including the pump, vacuum gauge, manifold, valves and needles for penetrating septa, as shown in the upper half of the manifold. The system shown is that used at the Agriculture, Food and Biosciences Institute in Northern Ireland (AFBI)



The angled, sharpened tip of this needle is called a 'regular bevel' by the manufacturer. A needle is inserted through each rubber septum into the vial and, along with a sharpened tip, the regular bevel angle has been designed to minimise the required force and drag. The bevel should not be longer than the thickness of the septum, otherwise air will enter when the vial is removed from the evacuation manifold. Care must be taken near needles to avoid personal injury (see Chapter of Health and Safety Considerations). Needles should be inspected regularly, because a damaged or worn bevel may cause septum damage. The evacuation rate becomes very slow after three or four minutes (Rochette and Bertrand 2008), and at NZ-NCNM, evacuation is done for five minutes. Alternatively, at AFBI, vials are evacuated to <100 Pa from a connected, pressurised tank, through a combination of evacuation and purging with helium, an inert gas. Rochette and Bertrand (2008) noted that the smaller helium molecule allowed more rapid and complete purging of vials.

The vacuum in a vial can be tested using a two-way needle, with one end inserted into a container filled with water, and the other penetrating a septum, and 'sipping' water into the vial before weighing it for comparison with the 'empty' weight (Rochette & Bertrand 2008). Rochette and Bertrand (2003) showed that vials with double-wadded septa could be evacuated up to 63 days prior to use, and that septa could be reused up to seven times. As shown below, each use may involve piercing a septum up to four times for (i) vial evacuation (ii) sample collection – including over-pressurising the vial (iii) sample equilibration to atmospheric pressure and (iv) sample analysis by the GC system. Results of the septa-piercing experiments reported by Glatzel and Well (2008) corroborated the earlier recommendation of Rochette and Bertrand (2003).

We recommend that an air sample be collected using a gas-tight polypropylene or glass syringe, connected to a gas-tight valve and needle (same specifications as above). To collect a sample, the needle is inserted through a rubber or silicon septum into the chamber headspace. The plunger is then pumped a few times to flush the syringe and any dead volume (de Klein *et al*. 2003). A sample can be transferred directly into an evacuated vial. The sample volume should be ~10 and ~20 mL for 6 and 12 mL vials, respectively. thereby over-pressurising the vial to minimise the incursion of ambient air during storage (~0.13% d^{-1}, Rochette & Bertrand 2003). The initial spontaneous movement of the syringe plunger prior to manual over-pressurisation of the vial is a useful visual indication of successful sampling. If the syringe plunger does not move spontaneously, it may indicate that the vial was not evacuated.

Once collected, the air sample must be stored until analysis, and the container should prevent sample loss until analysis. For samples with an N_2O concentration of 10 µL L^{-1} and storage periods of 14 and 126 days, between 92 and 98% of the original N_2O concentration could be recovered from containers using butyl rubber and doubled-wadded septa, respectively (Rochette & Bertrand 2003). Using butyl-rubber septa, for samples with an N_2O concentration of 1 µL L^{-1} and storage period of 365 days, 90% of the original N_2O concentration was recovered, and the decrease in N_2O concentration over time was linear (unpublished results from the NZ-NCNM laboratory). During storage, the external ambient N_2O concentration will be ~0.3 µL L^{-1}, and leakage rate

from the vials proportional to the concentration gradient, according to diffusion theory (Laughlin & Stevens 2003).

4.3 Gas chromatography

The purpose of a GC system is to separate N_2O from the other constituent gases in an air sample, and to reliably quantify its concentration. The detector of choice for N_2O is the 'hot' (>300°C) ^{63}Ni Electron Capture Detector (ECD), which is highly sensitive not only to N_2O, but other atmospheric gases, especially oxygen (O_2), water vapour, halogenated hydrocarbons (CFCs and freons) and carbon dioxide (CO_2).

The separation procedure has a physical basis. The detector operation, however, depends on the ionisation potentials and electron affinities of the compounds in the sample gas, the carrier gas and the ECD 'make-up' gas (see below). Ionisation potential is the energy required to remove an electron, and a common energy unit is the electron volt (eV), equivalent to 1.6×10^{-19} J. For example, the ionisation potentials of argon (Ar), N_2, CO_2, N_2O and methane (CH_4) are 15.8, 15.6, 13.6, 12.9 and 12.5 eV respectively, according to Zheng *et al*. (2008). Electron affinity is the energy released when a neutral gas species accepts an electron. This leads to the formation of negative ions.

At the NZ-NCNM, the entire 6 mL sample volume is injected into the GC, with the glass vial essentially taking the place of the fixed-volume sample loop normally employed for gas analysis, using more conventional GC injection procedures. Therefore, just prior to GC analysis, a sample's pressure is equilibrated to ambient atmospheric pressure, so the sample will have the same volume as the internal volume of the vial. This is done using a simple, inexpensive, double-ended needle device, similar to that described earlier, but here with an upwards-facing needle which can penetrate a septum cap, with the other end of the needle inserted into a container filled with water.

When the septum cap of an over-pressured vial is penetrated, the release of pressure produces bubbles in the water. When the bubbling stops, typically after two or three seconds, it is a visual indication that ambient atmospheric pressure has been reached. An alternative sample injection procedure exploits the overpressure in the vial to flush sample gas through a fixed-volume gas sample loop (typically 1 mL) which forms part of a gas-sampling valve (Hedley *et al*. 2006). Switching the valve – either manually or automatically – flushes carrier gas through the loop, and injects the sample onto the GC column.

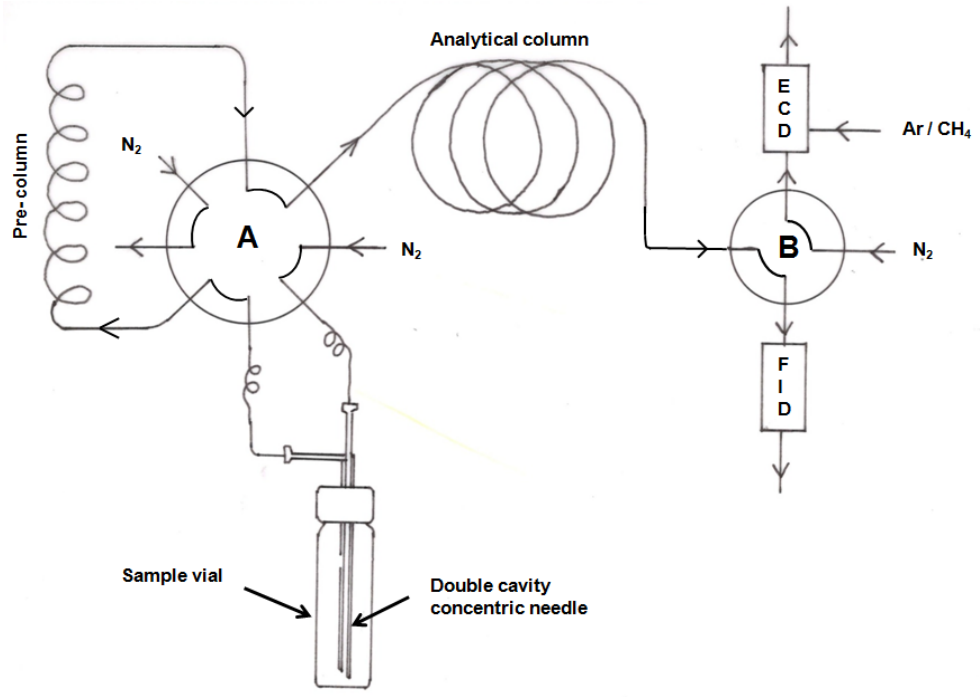
To optimise the precision and accuracy of results, it is strongly recommended that the GC analysis procedure be automated. By way of example only, we provide below details of the automated analytical procedure employed at the NZ-NCNM. Details of other automated procedures are available elsewhere (e.g. Zheng *et al*. 2008 and the references contained therein). At the NZ-NCNM, the procedure begins by placing the samples in Exetainers® into aluminium sample racks (capacity = 220 Exetainers® per GC system including samples and standards, manufactured by a local engineering firm), on the platform of an automated liquid 'handler' (model 222XL, Gilson, Inc., Middleton, WI, USA).

The sampling arm on the handler is fitted with a specially-constructed double-cavity concentric needle (Stevens *et al*. 1993). Such needles were originally designed to facilitate the injection of discrete gas samples into Isotope Ratio Mass Spectrometers, and are supplied through Sercon (Cheshire, UK). Needle movement is controlled by the handler's software, synchronised with a valve-switching sequence using software integral with the GC system (Peak Simple, SRI Instruments, Torrance, CA, USA).

At NZ-NCNM a typical analysis 'run' of 220 Exetainers® can be constructed with a block of 15 ambient samples and standards (sequence: 2x ambient air samples, then 11x N_2O standards, then 2x more ambient air samples), followed by blocks of 15 air samples separated by two ambient air or 0.3213 µL L^{-1} N_2O check samples. Standards for the purpose of GC system calibration span a range of N_2O concentrations, with samples from pressurised gas cylinders taken in the same way as the air samples, and transferred into vials. Finally, the run is completed by a second batch of 11 N_2O standards.

At NZ-NCNM, sample injection, pre-column 'backflush' (see later) and sample passage through the main analytical column to the ECD is controlled by two 10-port gas sampling valves, one of which is located at the head of the pre-column, while the other is located immediately prior to the ECD. The first 10-port valve (valve A) is actually configured as an 8-port valve, while the second 10-port valve (valve B) is configured to operate as a simple 4-port switch, whose main function is to direct the flow from the main analytical column either into, or away from, the ECD (Figure 4.2). This is essentially the same design first employed by Mosier and Mack (1980).

Figure 4.2: Simplified plumbing diagram, showing the gas sampling valves in the inject mode as described in the text, including the abbreviations. The system shown is that used at New Zealand's National Centre for Nitrous Oxide Measurement (NZ-NCNM)



But whereas the original Mosier and Mack (1980) GC system employed an Ar/CH_4 mixture as the carrier gas, most researchers now employ less expensive N_2. The carrier gas should ideally be oxygen-free, ultra high purity N_2 which, at NZ-NCNM (and elsewhere), is further purified by passage through a gas filter (Restek Corporation, Bellefonte, PA, USA) to remove any residual oxygen and water vapour. Separation begins with the N_2 carrier gas 'sweeping' the air sample via the double-cavity concentric injection needle, through a 1.5 mm ($^1/_{16}^{th}$ inch) outside diameter (OD) stainless steel transfer tube to valve A, and thence into the first of two $^1/_8^{th}$ inch (3 mm) OD GC columns, each packed with screened (125 - 149 µm diameter, analogous to fine sand), porous, resin beads (HayeSep D, a high purity divinylbenzene polymer, Valco Instruments Co., Inc., Houston, TX, USA).

The flow rate of the N_2 carrier gas (40 mL min^{-1}) can be controlled by an Electronic Pressure Controller (EPC). The GC system requires two other separate N_2 carrier gas flows, each of 40 mL min^{-1}. These flows are also controlled by their own EPCs. A fourth EPC can be employed to control the ECD 'make-up gas' (a mixture of 90% Ar and 10% CH_4) at 7 mL min^{-1}. Sample migration or elution time through a column also depends on its temperature, approximately halving for an increase of 30°C. However, among other factors, GC resolution generally decreases with increasing column temperature: minimal column temperature gives better resolution, but at the expense of a longer elution time. At the NZ-NCNM, a column temperature of 40°C has been found to be satisfactory, and the sample elution, or run, time is around eight minutes.

The GC systems employed at the NZ-NCNM operate in the same two modes as the system described by Mosier and Mack (1980). For the 'injection mode', during the first half of a sample's run, the N_2 carrier gas is flushed through the Exetainer® via valve A at 2.5 times the ambient pressure, which, as described above, injects the 6 mL gas sample onto a 1-m-long Hayesep D 'pre-column' (Figure 4.2). The main components in an air sample – N_2, O_2, CH_4, CO_2 and N_2O – all pass rapidly through this pre-column into the main 4 m-long analytical column, while the passage of the slower moving components – water vapour, CFCs and freons – is retarded.

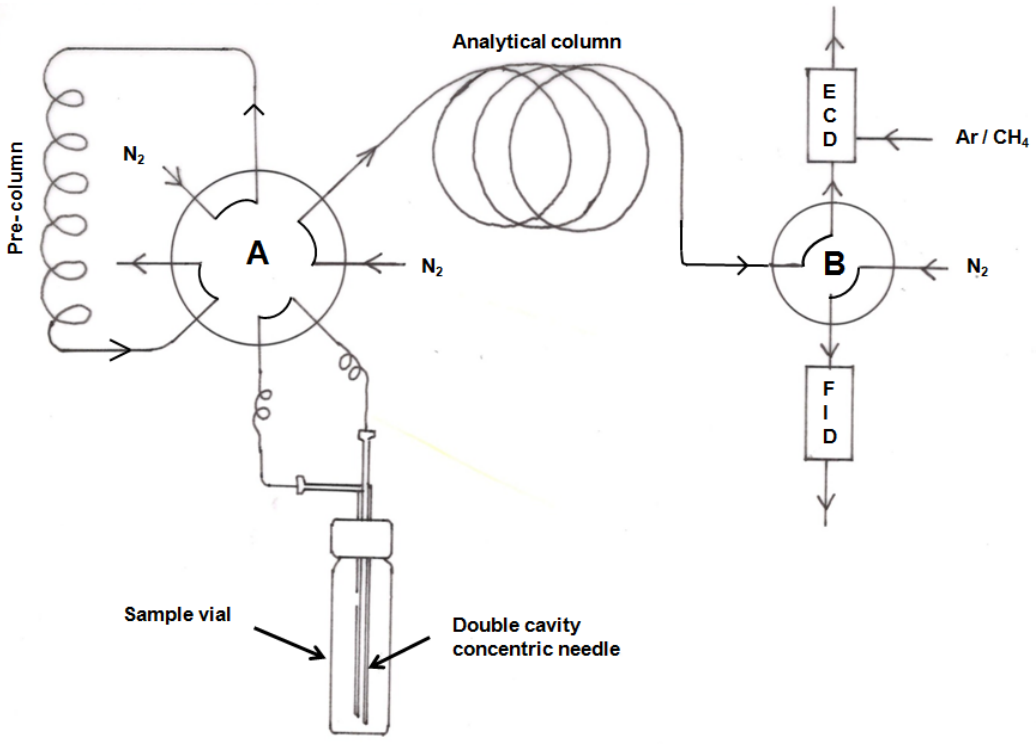
The O_2 in the gas sample has a very short elution time on Hayesep D. To prevent it passing into the ECD and overloading that detector, valve B, located at the posterior end of the analytical column, is switched to ensure that this large O_2 component elutes either through a flame ionisation detector (FID), or to waste. If this precaution is not taken, the response of the ECD to the more slowly eluting N_2O is compromised, making quantification more problematical (Zheng *et al*. 2008).

Repeated exposure of the ECD (operated at 310°C) to O_2 is also undesirable, as it is likely to shorten the life of the detector. The CH_4 in the air sample has a slightly longer elution time than O_2, but it can be quantified during this 'injection mode' if the effluent gas is permitted to exit the analytical column via valve B to an FID.

After about four minutes, during the last half of a sample's run time, both gas sampling valves are switched to a 'backflush mode' (Figure 4.3). During this period, the carrier gas flow through the 'pre-column' is reversed, the slow-moving compounds (see above) are vented to waste, and the flow from the analytical column is re-routed

through the ECD for quantification of N_2O. The N_2O elutes as a slightly non-Gaussian peak at 5.5 minutes following injection. At approximately 7.5 minutes, a separate command from the GC triggers the injection needle to move to the next Exetainer® in the analytical sequence, isolating that next sample for injection at eight minutes. This sequence is repeated, until all 220 samples and standards in a typical sample run have been analysed.

Figure 4.3: Simplified plumbing diagram, showing the gas sampling valves in the backflush mode as described in the text, including the abbreviations. The system shown is that used at New Zealand's National Centre for Nitrous Oxide Measurement (NZ-NCNM)



4.4 Electron capture detector

The ECD was first reported by Lovelock and Lipsky (1960). An ECD detects electronegative compounds, including N_2O. The ECDs employed at NZ-NCNM consist of a thermally-insulated, stainless steel cylinder encapsulating a radioactive source of Beta particles (^{63}Ni decaying at 185 MBq (5 mCi), SRI Instruments, Torrance, CA, USA). A sample's peak area depends on the temperature of the ECD, and at the NZ-NCNM, 310°C has been found satisfactory (Wentworth & Freeman 1973; Mosier and Mack 1980).

Beta particles (electrons) from the ECD collide with the N_2 carrier gas molecules, ionising them. This forms a stable cloud of free electrons, and when electro-negative

compounds, such as N_2O, enter the ECD, they combine with some of the electrons, temporarily reducing the number remaining in the electron cloud. Electron affinity enables the formation of negative ions. The detector electronics, which maintain a constant current (about 1 nA) through the electron cloud, then pulse at a faster rate to compensate for the decreased number of free electrons (Wentworth *et al.* 1966; Maggs *et al.* 1971; Wentworth and Freeman 1973).

The pulse rate is converted to an analogue output, becoming a measurement of the N_2O concentration, according to calibration of the GC system described next. The detector response is non-linear, and can be enhanced with a make-up gas. Di-nitrogen is not a particularly effective detection medium for N_2O using a ^{63}Ni ECD, but by introducing a more ionisable make-up gas separately into the ECD, the N_2O peak area can be enhanced. As noted earlier, for this purpose, we use a 90% Ar plus 10% CH_4 gas mixture at a flow rate of 7 mL min^{-1}. Moreover, using Ar/CH_4 as a make-up gas also eliminates a confounding effect of varying CO_2 concentration in the air samples, on determination of the N_2O concentration, as noted earlier by Zheng *et al.* (2008).

4.5 Calibration of gas chromatography systems

To accurately measure the N_2O concentration of an air sample, a GC system must be calibrated. As described earlier, each GC 'run' should include a similar range of standards ('reference' gases) and samples, and system performance can be further examined by including ambient 'checks' throughout the run. By way of example only, at the NZ-NCNM, calibration of each GC system involves 11 standards, each contained in a pressurised cylinder. There are 10 alpha-grade standards in G-sized cylinders comprised of a mixture of N_2O and di-nitrogen (N_2) gases. The alpha-grade, synthetic gas mixtures were prepared by a commercial gas-supply company in Auckland (BOC), and subsequently analysed to confirm their guaranteed 95% confidence interval (typically ± 1 - 2 % of the N_2O concentration), as well as their mean concentrations.

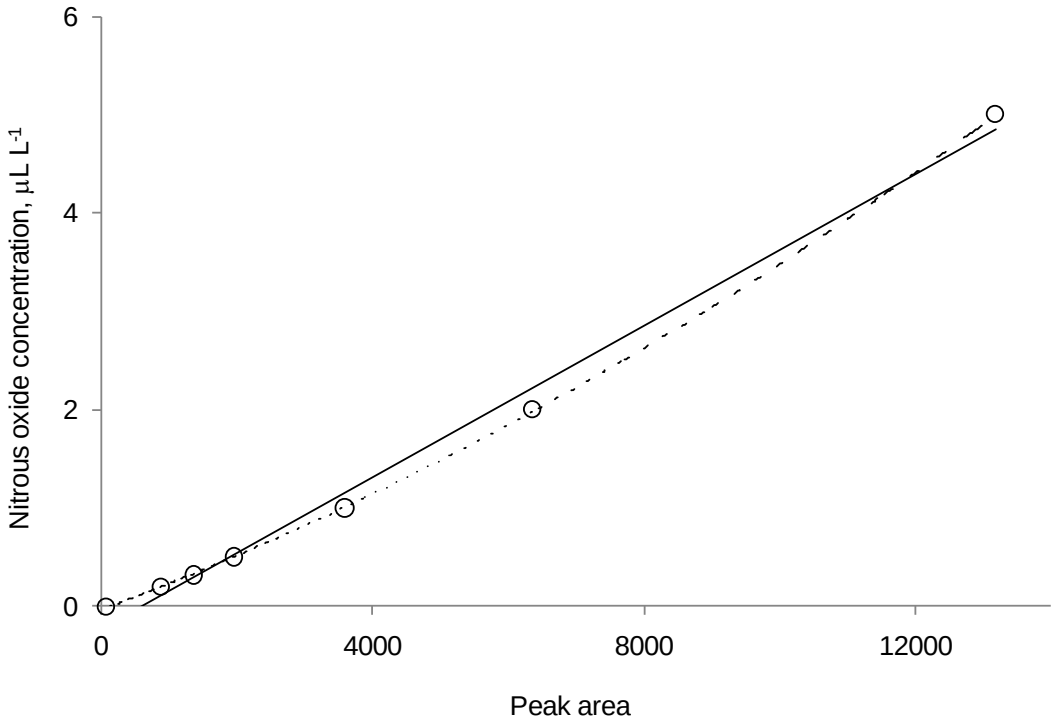
There is also a 'real' air standard. The first synthetic standard is pure N_2 gas, whose N_2O concentration is zero. The main purpose of this standard is to account for the very small quantity of N_2O from background lab air inevitably associated with the injection process itself, resulting in a very small positive N_2O peak area for that 'blank' N_2O standard (see Figure 4.4). The next standard has a sub-ambient N_2O concentration of 0.20 (±0.01) µL L^{-1}.

A real air standard was collected outside the NIWA (New Zealand's National Institute of Water and Atmospheric Research) laboratory at Greta Point, Wellington. The N_2O concentration of this air was measured by NIWA, using their GC system. NIWA's GC had been calibrated on the Scripps Institute of Oceanography SIO-98 scale, using standards certified by the National Oceanic and Atmospheric Administration (NOAA) Central Calibration Laboratory for GAW in November 2010 (Hall *et al.* 2007). A mean N_2O concentration for five samples of the bottled air from Greta Point was 0.3213 µL L^{-1} (± 0.0003 µL L^{-1}, this quantity being twice the standard deviation for 95% confidence, Gordon Brailsford, pers. comm.). The remaining eight synthetic standards had super-

ambient N_2O concentrations of 0.50 (± 0.01), 1.00 (± 0.01), 2.00 (± 0.02), 5.0 (± 0.1), 10.0 (± 0.2), 20.0 (± 0.4) and 50.0 (± 1) µL L^{-1}.

Inter-laboratory comparisons can be useful, and are recommended. This can involve the exchange of samples of a single concentration, as well as standards. For example, after five western Canadian laboratories exchanged air samples of a single concentration, as well as a 'reference' standard, reported means for the air sample ranged from 0.65 to 1.00 µL L^{-1} (Lemke *et al*. 2002). Using results for the reference standard, statistical analysis suggested a major source of the inter-laboratory variability could be attributed to the standards, different laboratories having had different commercial suppliers, evidently of variable quality.

Figure 4.4: The relation between peak area and N_2O concentration, determined by calibrating GC4 at the NZ-NCNM on 30 November 2011. On the basis of two regressions compared by an F-statistic, a line (solid) did not fit these data as closely as a quadratic curve (dashed, $p < 0.001$, N_2O concentration (µL L^{-1}) = -0.036 + 2.569 x 10^{-4} peak area + 9.544 x 10^{-9} peak area^2)



4.6 Processing gas chromatography data

To optimise the precision and accuracy of results, the GC analysis procedure can be automated. At the NZ-NCNM, the GC system software controls valve switching (Figure 4.2 and Figure 4.3) and sampling rate (1 Hz). The software also records and integrates analogue signals from the ECD and FID, and produces the chromatograms on the controlling computer's screen. After analysis of each batch of samples, these post-run

chromatograms are scrutinised, and baseline correction and adjustment of 'integration windows' applied if necessary. An integrated results file can then be exported to Excel® for further post-run processing.

That processing begins with the standards; determining a relationship between peak area and N_2O concentration for the GC system. While all standards should be included in each GC run, a selection may be more appropriate for determining the relationship, depending on peak areas of the samples. To illustrate by example, to calibrate a GC, seven standards were selected, including N_2O concentrations of 0, 0.200, 0.3213, 0.500, 1.00, 2.00 and 5.00 µL L^{-1} (Figure 4.4). This GC run included one sample with an N_2O concentration < 0.3213 µL L^{-1} (the minimum with 0.320 µL L^{-1}), 43 between 0.3213 and 0.500 µL L^{-1}, 25 between 0.500 and 1 µL L^{-1}, 15 between 1 and 2 µL L^{-1}, and six between 2 and 5 µL L^{-1}, with a maximum value of 3.2 µL L^{-1}. Linear and quadratic (second-order polynomial curve) models were fitted to these data by regression methods. Using an F statistic, the two (nested) models were compared, to indicate which curve fitted the data significantly better than the other: a quadratic curve fitted these data more closely than a line. Generally, a quadratic curve has been used for GC calibration.

Statistics can also be calculated to determine GC performance. To illustrate some principles following Ellison *et al*. (2009), a repeatability standard deviation, SD_r, can be calculated in the usual way from results obtained using the same method and a set of replicate air samples from the same source, under the same conditions – operator, GC system and laboratory. This statistic, quantifying within laboratory variability by a square root of the within laboratory variance, SD_{wi}^2, may be written:

$$SD_r = \sqrt{SD_{wi}^{\ 2}} \tag{1}$$

A reproducibility SD, SD_R, applies to results using the same method, and sets of replicate air samples under different conditions (different operators, GC systems and laboratories). This statistic quantifies total variation, calculated by combining the between-laboratory variance – SD_{be}^2 – the sample/laboratory interaction (if it exists) and SD_{wi}, and it may be written:

$$SD_R = \sqrt{SD_{be}^{\ 2} + SD_{wi}^{\ 2}} \tag{2}$$

Repeatability – r – is the value below which an absolute difference between two single test results – obtained with the same method, on sets of replicate air samples under the same conditions (operator, GC system, laboratory and a short interval of time) – may be expected to lie. This may be written:

$$r = t * \sqrt{2} * SD_r \tag{3}$$

where t is the Student t, two-tailed value, for n - 1 (n, number of replicates) for a given confidence, usually 95%. Finally, reproducibility – R – is the value below which an absolute difference between two single test results, obtained with the same method,

on sets of replicate air samples, under different conditions (operator, GC system, laboratory and/or different times) may be expected to lie. This may be written:

$$R = t * \sqrt{2} * SD_R \quad (4)$$

A worked example of procedures for determining the precision of determination of ambient concentrations of N_2O is detailed in Appendix 2.

4.7 Relating N_2O sample analyses to N_2O fluxes

For measurements of N_2O fluxes by the chamber method, the variables will include: the height of the soil chamber head space; the interval between gas samples taken from the head space; and SD_r of the GC system. The GC system's precision will quantify the variability of measurements for a set of samples, each having the same N_2O concentration. In essence, the smaller the variability, the greater is the signal-to-noise ratio of a GC system.

To illustrate the determination of SD_r for a GC system, we will utilise the data of 19 January 2011, for GC4 at the NZ-NCNM. There were four sets of 20 test samples, with N_2O concentrations of 0.200, 0.3213, 0.500 and 1 µL L^{-1}, as well as a fifth set of 20 air samples obtained from a field trial site. The fifth set emulated background air samples collected during N_2O flux measurement field trials.

We investigated whether or not the precision of the five sets of samples was affected by the N_2O concentration. This was based on a premise that precision of an ECD might be affected by the density of N_2O molecules in an air sample. We included samples from three synthetic standards (0.200, 0.500 and 1.00 µL L^{-1} N_2O in N_2), as well as samples from the real air standard (0.3213 µL L^{-1}) in order to investigate whether or not the precision of synthetic samples would be different to that of real air. As stated, our synthetic samples consisted of N_2O diluted into N_2, and did not include the other gases in air, with O_2, CO_2 and H_2O of particular relevance in this context.

The five sets of 20 samples were analysed, as well as fifteen check samples containing real air collected outside the NZ-NCNM laboratory, and ten calibration standard samples. The total of 125 samples had been placed randomly in the auto-sampler racks. Each sample took eight minutes to analyse, so 125 samples were analysed over a period of 16.7 ≈ 17 hours. Precision was quantified by analyses of the sample peak areas, so the GC system calibration was not involved. A coefficient of variation (CV, %) can be computed by SD_r expressed as a percentage of the mean N_2O concentration for a set of samples.

Results showed that the CV for synthetic N_2/N_2O mixes containing 0.200 and 0.500 µL L^{-1} N_2O were identical at 1.7%, but 21% larger (CV = 2.06%) for synthetic mixes containing 1 µL L^{-1} N_2O. These data suggested the CV was not consistently, nor significantly, affected by N_2O concentration of the synthetic mix samples analysed. While the samples used for this determination were either synthetic N_2/N_2O mixes or real air samples, additional analysis indicated the CV was not influenced by the origin of the sample. Likewise, a comparison of CVs for real air from Greta Point sampled from the 0.3213 µL L^{-1} bottle (CV = 1.07%), and real background air sampled at a trial

site (CV = 1.25%) suggested real air samples from a trial site varied about the same as a set of real air samples from a pressurised bottle.

We compared the CVs obtained at the NZ-NCNM to that of overseas laboratories, based on reports published in peer-reviewed journals. For a USDA laboratory at Fort Collins, Colorado, ten measurements of air with a mean N_2O concentration of 0.316 µL L^{-1} had a standard deviation of 0.001 µL L^{-1}, so the CV for their GC measurement system was 0.32% (Mosier and Mack 1980). Similarly, for the University of Edinburgh laboratory, twenty measurements of air with a mean N_2O concentration of 0.316 µL L^{-1} had a standard deviation of 0.00132 µL L^{-1}, so their CV was 0.42% (Arah *et al*. 1994). Finally, for a CSIRO laboratory at Aspendale, Melbourne, Australia, the GC measurement system precision of replicate analyses was reported to be 0.3%, and while the statistic was not specified, it was assumed this was a ratio of SD_r expressed as a percentage of the mean N_2O concentration (Galbally *et al*. 2010).

The CV for GC4 was different to that of three overseas laboratories. A different feature of the GC systems employed at NZ-NCNM is that the sample volume is determined by the volume of the sample container – the vial. As such, the air sample volume is intended to be 6 mL. As a component of the CV for this GC system, volume variability from one vial to another corresponds with variability in air sample volume. A GC detector responds to the number of N_2O molecules. Air includes N_2O, so for a given N_2O concentration, a sample of greater volume will include more N_2O molecules than a sample of lesser volume.

Here, we present an illustration from the NZ-NCNM system of the impact of measurement system precision on detectability of flux. To examine the variability of sample volume, volumes were measured for a set of vials by an Archimedes method. A vial was carefully filled with water – as judged by the meniscus that formed at the top (opening) of the vial – and weighed inside a closed, glass cabinet on a sensitive (1 mg resolution) balance. To estimate the reliability of this method, the volume of a single vial was repeatedly measured nine times, yielding a CV of 0.27%. Measuring volumes of a set of 20 vials yielded a CV of 0.87%. To further check the method, a different person measured the volumes of different sets of 14 and nine vials, and the corresponding CVs were 0.77 and 0.68%, respectively. Broadly similar results (CVs) were obtained by two people, and the average CV was 0.77%. This included a method (repeatability) error quantified by the CV of 0.27%. To eliminate the method error, a root mean square calculation was done, yielding the variability of sample volume quantified by a CV of 0.72% (= $\{[0.77^2] - [0.27^2]\}^{0.5}$).

The variability of GC measurements of real air samples from a trial site was quantified by a CV = 1.25%. One component of this GC precision may be attributed to the variability of air sample volume. Measurements indicated this component of the GC precision was quantified by a CV of 0.72%. Combining, we can deduce that the variability of air sample volume (i.e., the 'sample loop') was responsible for about half the variability of the GC measurements of real air samples (=0.72%/1.25%).

To move on to the connection between SD_r for a GC system and N_2O fluxes, we write a simplified equation for N_2O flux calculation measured by the chamber method as:

$$F_{N2O} = (\partial c/ \partial t)*(M/V_m)*(V/A) \quad (5)$$

where ∂c is the change of N_2O concentration in the chamber headspace during an enclosure period (µL L^{-1}); ∂t the enclosure period (h); M the molar mass of N in N_2O (g/mol); V_m the molar volume of gas at the sampling temperature and atmospheric pressure (L/mol); V the headspace volume (m^3) and A the area covered (m^2). The headspace height is (V/A). For more detail of chamber flux calculation methods, see the Data Analysis Considerations Chapter. For analysis, we will assume ∂c has been determined by c_e and c_b – N_2O concentrations at the beginning and end of an enclosure period – in order that we can also write:

$$F_{N2O} = U*(c_e - c_b) \quad (6)$$

where U subsumes the other terms in equation (5). Using equation (2), we will write an equation for the SD of F_{N2O} as:

$$SD[F_{N2O}] = U*(SD[c_e]^2 + SD[c_b]^2 - 2*SD[c_e]*SD[c_b]*\rho)^{0.5} \quad (7)$$

where ρ is the correlation between c_e and c_b. For illustration, we will assume SD_r of a GC system determines SD[c_e] and SD[c_b], so SD[c_e] = SD[c_b]. On this basis, using equation (7), we calculate a lower limit for SD[F_{N2O}] of 0 when ρ = +1, its maximum value. Further, SD[F_{N2O}] increases as ρ decreases, reaching an upper limit when ρ = -1, its minimum value. When ρ = 0, we will write c_a as the average of c_e and c_b, so equation (7) can become:

$$SD[F_{N2O}] = U*2^{0.5}*SD[c_a] \quad (8)$$

Repeatability in gas measurement should not limit flux detection capability. It is recommended that error in flux estimate due solely to laboratory repeatability should be at least an order of magnitude smaller than fluxes being measured. To illustrate how the equations connect SD[c_a] (estimated by SD_r of a GC system) and SD[F_{N2O}], an estimate of the smallest N_2O flux that can be reliably measured, some example calculations will be done. For term U, we will set ∂t = 0.33 h, chamber height = 0.1 m and diameter = 0.5 m so that [V/A] = 0.1 m, atmospheric pressure = 1 (Atm), air temperature = 10°C at sampling, and units of F_{N2O} and c_b and c_e equal to µg N m^{-2} h^{-1} and µL L^{-1}, respectively. Thus, for our calculations, U will be 365 µg N m^{-2} h^{-1}µL^{-1} L.

We recognise that if ∂t alone increased, U would decrease proportionally, meaning SD[F_{N2O}] would also decrease proportionally according to the (unchanged) value of SD[c_a]. Alternatively, if [V/A] alone increased, U and SD[F_{N2O}] would also increase proportionally. To proceed further, we set SD[c_a] to 0.004 µL L^{-1}, reflecting a CV of 1.25% for real air samples, recognising that this does not include any error associated with the calibration curve. For our calculations, the mean value will be 0.3213 µL L^{-1}, and SD[c_e], SD[c_b] and SD[c_a] will be 0.004 µL L^{-1}. To illustrate potential effects of ρ on SD[F_{N2O}], we will do calculations as a sensitivity analysis. For these calculations, ρ will be ≤ 0, meaning c_e is inversely proportional to c_b. A basis for this assumption can be a diffusion argument, by which F_{N2O} would be proportional to the soil-to-chamber N_2O concentration gradient. In this situation, if c_b alone increased, F_{N2O} decreases, so c_e must also decrease according to equation (2).

As a baseline calculation, when ρ = 0, by equation (4), SD[F_{N2O}] will be 2.07 µg N_2O-Nm^{-2} hr^{-1} (= 365*$2^{0.5}$*0.004). Compared to this value of SD[F_{N2O}], when ρ = -0.1, -0.2, -0.4, -0.6 and -1, by equation (3), SD[F_{N2O}] will be 5, 10, 18, 26 and 41% larger, respectively. Thus, using a diffusion argument for ρ, the effect of ρ on SD[F_{N2O}] should be minimised by constraining c_b to the background or ambient level, which can be checked by the GC analyst. Such calculations also illustrate the merit of accurate and precise N_2O sample analysis for the determination of N_2O fluxes by the chamber method.

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5 AUTOMATED GREENHOUSE GAS MEASUREMENT IN THE FIELD

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Summary table

Table 5.1: Summary of considerations for deployment of automatic systems

Overarching issue	Deployment issue	Objective	Minimum requirements	Site or situation specific issues	Evolving issues
Design	Light for plant growth.	Adequate photosynthetically active radiation (PAR).	Stainless steel frames and transparent acrylic panels.	Consistent high temperature situations may require tinted acrylic, which has a minimal impact on PAR during chamber closure. In hot environments, a reflective surface on the top of the chamber may be required.	
	Gas leaks.	Prevent leaks during chamber closure.	Non-reactive adhesives to form a tight seal.	Extreme temperatures may require the use of specialised adhesives.	
	Pressure changes.	Reduce artifacts associated with chamber closure and sampling.	2-3 mm vent (this is normally of the same design as the gas sample port).		
	Representative gas sample.	Avoid gas sample gradients within chambers.	A single sampling port at the top of the chamber is sufficient in volumes < 50 litres.	If extensions are used, fit multiple sampling ports.	Mixing fans.

Overarching issue	Deployment issue	Objective	Minimum requirements	Site or situation specific issues	Evolving issues
Individual chamber deployment	Site disturbance.	Minimise effect of site disturbance on flux estimate.	Two chamber bases per replicate plot, to be inserted at least 24 hours prior to the first sampling. Switch chamber position between bases every week.	In a cracking clay soil under dry conditions, ensure the seal between the soil and the inner and outer walls of chamber base is filled with additional soil from the plot.	
	Chamber air temperature.	To minimise heating artifacts created during chamber closure	Air temperature sensor within at least one chamber (in a block design) to trigger opening at a pre-defined temperature (<50 deg C).	Temperature threshold is dependent on specific plant tolerance to heat. Use tinted panels or insulation in high temperature environments.	
	Precipitation and rainfall.	Ensure all chambers receive the same amount of water.	Rain gauge, with threshold set to open all chambers.	Thershold for opening chambers will depend on local conditions, and can be based on depth (e.g., 2 mm) or on rate (e.g. 0.4 mm/h).	
	Chamber sequence.	To avoid bias due to sampling order or pattern.	Ensure that measurements are sampled per block, rather than per treatment.	The proximity of the individual chambers to the sample analysis equipment depends on flow rates (e.g. < 70 metres maximum in the case of the Queensland auto-systems).	

Overarching issue	Deployment issue	Objective	Minimum requirements	Site or situation specific issues	Evolving issues
	Ancillary measurements	To help explain findings, to develop functional relationships and/or determine proxy measurements.	Profile soil texture, bulk density, pH, total C and total N content at least once per season. Soil sampled for mineral N (0-10 cm) every month, deeper increments preferred). Weather station nearby. Sensors for continuous logging surface soil and air temperature, soil moisture.	Depth of sampling is dependent on local conditions and resources.	Researchers to consult with modellers on additional model input parameters requiring measurement and at what frequency.
Equipment	Power, weather proofing and security.	Reduce risk of sample analysis equipment failure and loss of data.	Either trailer or shed with access to mains power within 100 metres, including UPS, or remote power source: e.g., solar, with back-up generator.	Solar with batteries is possible with GC systems (refer U. Sydney).	
	Calibration and carrier gases	Minimise disruptions to continuous sampling	Spare gas cyclinders and calibration gases.	If extended downtime, use manual sampling procedures	

Overarching issue	Deployment issue	Objective	Minimum requirements	Site or situation specific issues	Evolving issues
Quality control	Leaks or blocks in chamber or sampling line.	Minimise poor quality data.	A Licor CO_2 analyser will provide high temporal resolution data which can provide a rapid graphical assessment of leaks. Ensure ascarite (H_2O absorber) is regularly changed. Regular visual inspection of chambers and sampling lines.	Where rodents are present, shield gas sampling lines in tubing. Fence livestock away from sample lines and auto-chambers.	
	Data quality and continuity.	Reduce risk of poor data, or data loss.	Regular visual assessment of graphical outputs. Regular computational analysis of data. Computer backups.	Remote access wireless or internet communication for 24/7 checking.	

5.1 Introduction

In addition to the manual chamber systems described in previous chapters, N_2O emissions can also be measured using automated systems, which collect and analyse greenhouse gases in real time in the field. The basic requirements of chamber design – and the need to minimise soil, plant and environmental disturbance – are identical to those for static chambers, and are discussed in more detail in Chapter 2. This chapter covers additional aspects and requirements specific to automated systems.

Previous efforts to automate chamber-based measurements of greenhouse gas emissions (e.g. Denmead 1979; Christensen 1983a; Ambus & Robertson 1998) relied on near-continuous flow systems. However, in the past decade, more emphasis has been placed on the modification and automation of the static chamber, non-steady state, non-flow-through technique. Automation allows the measurement of fluxes after rain, irrigation or other disturbances when manual chambers would be difficult to access or use. It can also reduce soil disturbance, which can be an issue with manual sampling, especially during difficult conditions.

Automation also allows for detailed assessments in remote locations (e.g. savannah woodlands of northern Australia, Livesley *et al*. 2011), where manual sampling might be uneconomic. Automated chambers are considerably more expensive than manual chambers, but they can be used in conjunction with manual chambers in order to characterise temporal variability. This can be a useful approach in experiments where a large number of treatments are compared, and where it is impossible to deploy large numbers of auto-chambers.

Automated chamber systems can include an in-field gas analyser or, alternatively, gas samples can be automatically stored in vials, inert gas sampling (foil) bags or sample loops and analysed later in the laboratory (e.g. Ambus *et al*. 2010; Smith & Dobbie, 2001). The cost of automated equipment is highly dependent on the choice of the gas analysis technology, operational costs and system configuration (e.g. with or without in-field analyser). Gas chromatography is the cheapest option, with a system with 12 chambers and an in-field analyser costing around USD$100,000.

5.2 Diurnality

Diurnality (time of day) in the context of manual chambers and sampling has been discussed in Chapter 3. One of the main advantages of automated sampling is the ability to capture diurnal fluctuations in emissions – a laborious task when repeated manual sampling is undertaken over a 24 hour period. Diurnal variations in soil-derived N_2O, CH_4 and CO_2 emissions are largely due to temperature variation (Christensen 1983b; Sass *et al*. 1991; Maljanen *et al*. 2002; Savage and Davidson 2003). N_2O fluxes are generally higher during the day, and increase exponentially with soil temperature (Flessa *et al*. 2002). Temperature sensitivity is also moisture-dependent, with the rate of change greatest when soil moisture level approaches field capacity (Meyer *et al*. 2001).

Several studies have found a close relationship between N_2O fluxes and surface soil temperature, but others have found a lag of several hours between maximum flux and maximum temperature. For example, using automated chambers, Scheer *et al*. (2012) found that the diurnal variation of N_2O fluxes from sub-tropical irrigated wheat was greater than 10-fold for some chambers, with maximum emissions between 1800 and 2400 hours and minimums between 0800 and 1400 hours. Land use must also be taken into account.

Rowlings (2010) disaggregated two years of high temporal resolution, comprising 10 automated samples per day for adjacent land uses in subtropical Queensland. He found that errors arising from single daily calculations of average daily N_2O flux rates could be minimised if rainforest, pasture and lychee (tree crop) sites were sampled in the morning, at noon and in the afternoon. Diurnal patterns may not be consistent throughout the year (Du *et al*. 2006; Yao *et al*. 2009), and can be obscured by dry and/or wet conditions causing rapid anoxic conditions, which stimulate N_2O production via denitrification (Savage and Davidson 2003). Similar diurnal trends are also observed with CH_4 emissions from rice systems (Buendia *et al*. 1998).

5.3 Sample frequency

The highly episodic response of N_2O emissions to changes in soil water status, and the availability of labile sources of carbon and nitrogen fertilisation, means sampling frequency throughout the year or season has a profound effect on the calculation of cumulative emissions. A major limitation of a manual chamber sampling strategy is that it cannot adequately handle the impact of climatic variability: potentially large emission pulses are not captured. The high temporal frequency of automated measurements greatly improves their ability to measure (and ultimately predict) the effects of rapidly changing soil water content on emissions, and their interaction with management events such as fertiliser applications (Savage and Davidson, 2003).

A number of field experiments have compared manual and automated chamber methods (e.g. Ambus *et al*. 2010; Smith and Dobbie 2001). However, all suffer from the difficulty of having to compare different techniques in different places or at different times, thus confounding the consideration of technique with either spatial or temporal heterogeneity. Rowlings (2010) examined the impact of sampling frequency on the accuracy of annual flux estimates across multiple land uses (Table 5.2). At sampling intervals of three days or fewer, errors associated with sampling frequency were greater than the diurnal variability of N_2O emissions, suggesting *sampling interval is more critical than sampling time*.

At weekly intervals or greater, errors increased significantly, potentially overestimating emissions by over 100% for agricultural land uses. In all land uses, coarser sampling intervals tended to overestimate, rather than underestimate, cumulative emissions estimates. Van der Weerden *et al*. (2013) conducted three short-term field trials where N_2O fluxes were measured from urine-affected pastoral soil, and bias in cumulative emissions created by infrequent sampling was assessed. They recommended that gas be sampled twice a week between 1000 and 1200 hours over the first four to six weeks

after urine deposition, with additional sampling after significant rain (e.g. > 10mm/d). This sampling regime produced a +4% bias, compared to cumulative emissions based on frequent, two-hourly, flux measurements. It was therefore considered to represent a balance between practicality and data quality for estimating cumulative emissions. Interestingly, Yao *et al*. (2009) report that intermittent manual and continuous automated measurements would result in comparable CH_4 fluxes in non-waterlogged soils.

Scott *et al* (2000) compared auto-chambers with manual chambers in measurements of N_2O emissions from grassland receiving either sewage sludge or synthetic fertiliser N. The manual chambers were sampled up to twice per week, and the auto-chambers were sampled up to six times per day over a six month period. The study found that cumulative emissions from the sludge treated plots over the six-month period measured by auto-chambers was 20.6 kg N_2O-N ha^{-1}, while that from the manual chambers was 13.3 kg N_2O-N ha^{-1}. However, these differences were not significant, due to a high coefficient of variation (27.4%), and it was interesting to note that the manual chambers were better at identifying treatment effects, because the increased replication of the manual chambers was able to take account of the spatial heterogeneity.

A variant of the auto-chamber technique has been reported by Ambus *et al*. (2010) (Figure 5.1). They compared manual chambers with two different auto-chamber systems. In the first, samples were collected and stored in sample vials as described above, but in a variant of the auto-sampler approach (SIGMA), samples were collected and stored in foil bags. Samples collected on each occasion were added to the previous sample. The advantage of this system is that it provides an accurate assessment of cumulative gas fluxes over time, with significantly lower analytical costs. Their study found no significant differences in the accumulation of N_2O in standard auto-chambers, manual chambers and SIGMA auto-chambers, and all three approaches were able to capture aspects of temporal variability in fluxes, but at different time scales. Again, spatial heterogeneity was considered an important explanation of the differences observed.

Figure 5.1: A comparison between N2O fluxes measured from a grazed grassland in Scotland, using manual chambers and an autochamber. Data taken from a study by Ambus *et al*. (2010)

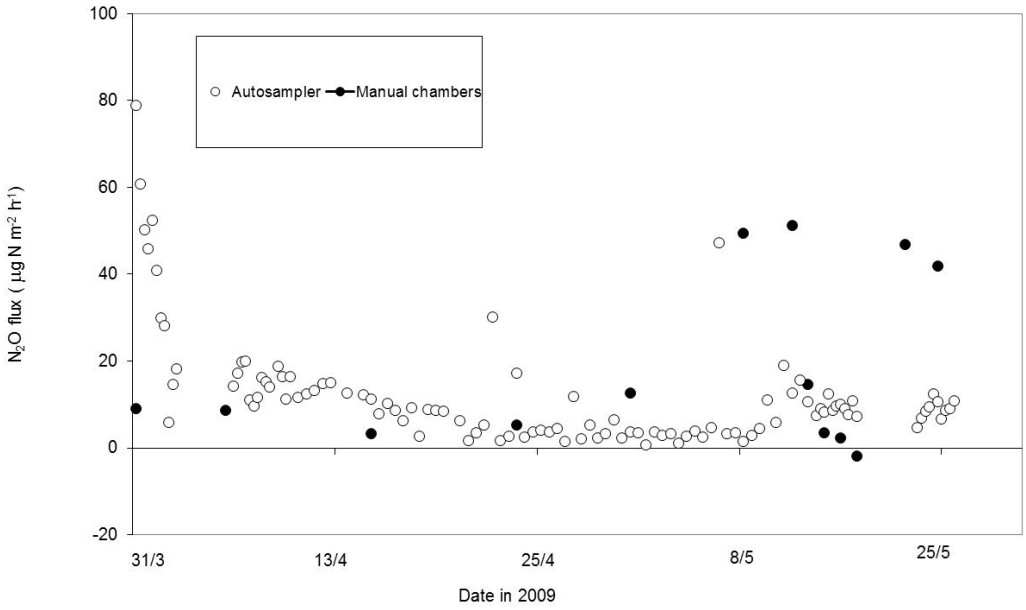


Table 5.2: Maximum and minimum deviation from annual N_2O fluxes (% deviation from mean) from three land uses in sub-tropical Queensland, using different sampling frequency permutations (Rowlings, 2010)

Land use	Sampling frequency (days)						
	1*		2	3	7	14	30
Rainforest	516	Min	-3	-4	-16	-19	-34
		Max	3	2	15	26	183
Pasture	1827	Min	-3	-10	-22	-32	-53
		Max	3	16	30	32	183
Lychee	1712	Min	-2	-2	-34	-48	-67
		Max	2	3	28	58	108

**Annual estimate of N_2O emissions (g N/ha/annum) using a fully automated greenhouse gas measurement system with 10 cycles per day over 2 years.*

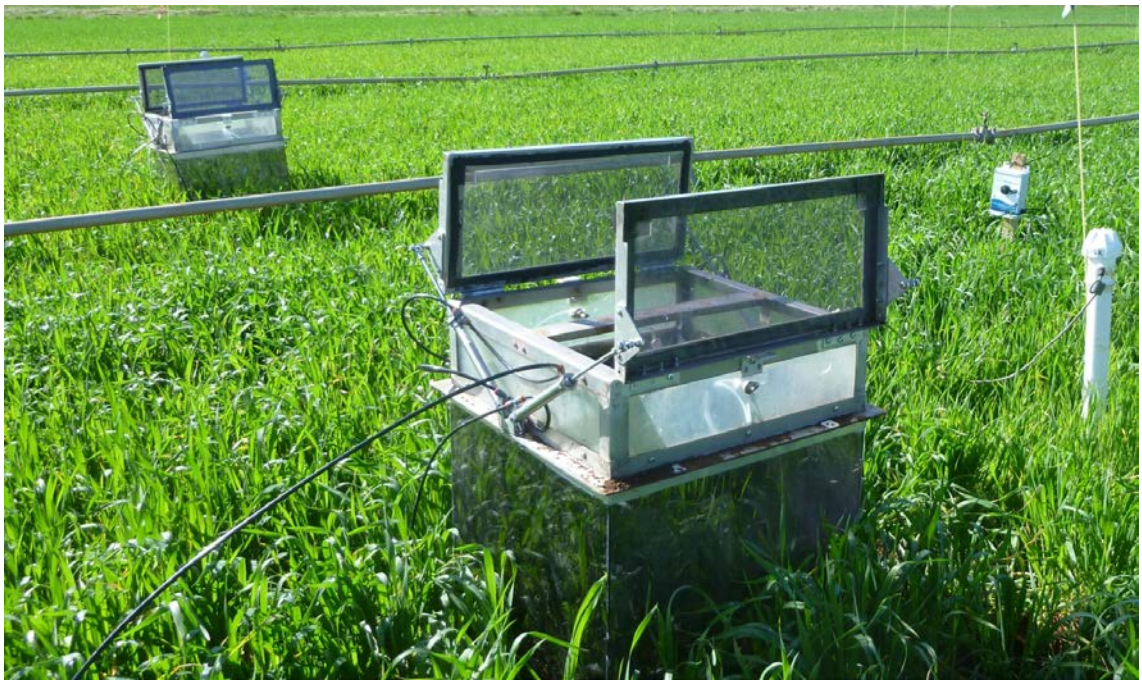
5.4 Operating principles

The basic principle of current automated systems is to utilise the static closed chamber technique (non-steady-state, non-through flow) to capture nitrous oxide (N_2O), carbon dioxide (CO_2), and methane (CH_4) fluxes from soils. The system may consist of numerous automated chambers linked to a sampling system, and an in-situ gas chromatograph (GC) (Kiese *et al*. 2003; Rowlings *et al*. 2012; Scheer *et al*. 2011), tuneable diode laser (TDL) (Officer *et al*. 2012), Fourier transfer infrared (FTIR)

spectrometer (Kelly *et al*. 2008) or photo-acoustic infra-red spectroscopy (Yamulki and Jarvis 1996; van der Weerden *et al*. 2013) for N_2O, CH_4 and CO_2, or a subset of these gases. In some cases, an infrared gas analyser is used for CO_2. Alternatively, the auto-sampler may be used to transfer gas samples to vials in the field, which are subsequently analysed using standard gas chromatography methodologies (Scott *et al*. 2000; Ambus *et al*. 2010).

External sensors also collect high-resolution environmental data, including soil and air temperatures, soil moisture and precipitation. Fluxes are derived by measuring the increase or decrease in gas concentration inside the chambers' headspace over the closure time (normally 30 to 60 minutes). In total, each chamber is sampled repeatedly (normally a minimum of four times) over the defined closure time, allowing multiple flux rates to be measured for each greenhouse gas chamber per day. Gas concentration in an enclosed atmosphere can build up to levels which may inhibit normal emission rate (Buendia *et al*. 1998). A closure period of up to 30 min is considered acceptable for CO_2 (Kessavalou *et al*. 1998), whereas the optimum for N_2O can be longer (Jury *et al.* 1982).

Figure 5.2: Automated chambers developed by Queensland University of Technology (Australia) in collaboration with Karlsruhe Institute of Technology (Germany). In this picture, standard 37.5 litre chambers are atop 125 litre extensions to accommodate wheat

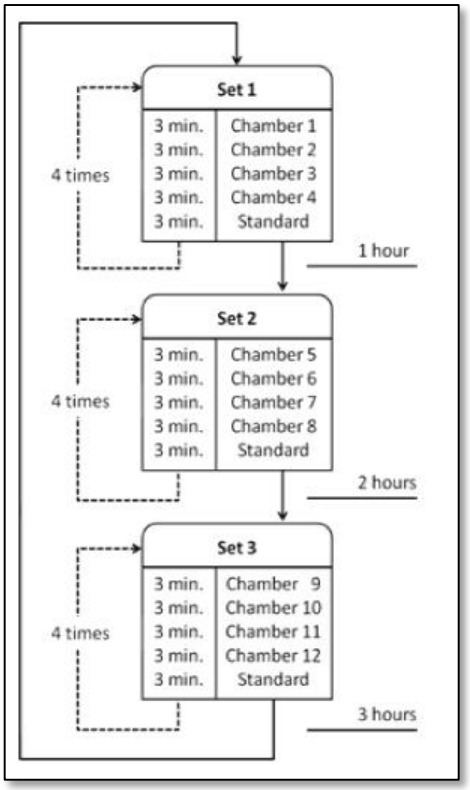


The following is a working example of one of the more popular automated sampling systems, which consists of twelve 37.5 litre chambers (Figure 5.2), allowing multiple treatments to be assessed at a single location. This unit is based on the original system as described in Kiese and Butterbach-Bahl (2002), and used extensively in Australia (Barton *et al*. 2008; 2010; Rowlings *et al*. 2012; Scheer *et al*. 2011; 2012; Wang *et al*.

2011), including the national Nitrous Oxide Research Program (Grace *et al*. 2010). The modified system, which we will call the 'Queensland' system, is now produced by Queensland University of Technology in collaboration with Butterbach-Bahl (Karlsruhe Institute of Technology). This system has also been deployed in the USA (Michigan State University), Chile (INIA) and India (ICRISAT).

The normal layout is three sets of four chambers, of which one set is sampling at any one time. This allows for up to four treatments to be sampled simultaneously; each replicated three times. Air samples are taken sequentially from each closed chamber, followed by a calibration standard (i.e., after every fourth sample). In total, each chamber is sampled four times (every 15 minutes) over 60 minutes. When the current set of chambers open, the next set of four chambers closes, and begins the sampling sequence (see Figure 5.3). It takes 180 minutes for all chambers to be sampled in one measurement cycle.

Figure 5.3: A twelve-chamber sampling sequence with four treatments



Fluxes are calculated from the slope of the linear increase or decrease in N_2O concentration during the chamber lid closure, then corrected for air temperature, atmospheric pressure and the ratio of chamber volume to surface area, as described in detail by Barton *et al*. (2008). The Pearson's correlation coefficient (r^2) for the linear regression is normally calculated and used as a quality check for the measurement. Flux rates were discarded if r^2 was < 0.80. More detail on flux calculations and correlations can be found in Chapter 6. A single automatic chamber produces up to 3000 flux estimates per year, which ensures significantly increased temporal accuracy compared to manual chamber approaches. The experience of the many users of these

automatic systems within the Australian Nitrous Oxide Research Program is that the vast majority of emissions are linear, with an $r^2 > 0.9$ in a well maintaining, functioning auto-system.

The portable automated greenhouse gas measurement comprises two main parts: the automated chambers and the sampling unit, which also includes software to run the system and record data. Chamber operation, sampling, and data acquisition are computer-controlled and run continuously. Mains power or a generator is required.

5.5 Chamber design

The Queensland automated chambers are sealed airtight during gas sampling by two lids, which open and close via pneumatic actuators (with air supplied from a compressor). The standard chambers (500 mm x 500 mm x 150 mm) are stainless steel frames, with transparent acrylic panes which have external outlet and inlet ports (1/8") – the latter a vent to ensure equalisation of pressure during gas sampling (Hutchinson and Livingston 2001). The outlet port is connected to an internal stainless steel sampling line with multiple holes, which extends to the centre of the chamber. Extensions of either 300 mm or 500 mm can be fitted to raise the height of the chambers for use with a variety of agricultural crops.

Ideally, the plants in the chamber will mimic the surrounding environment as closely as possible. However, this can introduce artefacts associated with plant effects, (see Chapter 2.8) and elevated CO_2, although this can be minimised by shifting the chamber position regularly during the season. Neither is it practicable to enclose tall crops, such as maize or sugar cane, so chambers are normally placed between the rows.

A second example of a working system is one developed by AgResearch in New Zealand (Figure 5.4), based on that reported by Ambus and Robertson (1998). This system (termed the AgResearch system) resembles the Queensland system in many aspects. However, the design and construction of the nine chambers differs, with the use of linear motors to move a one-piece lid into the closed position, where electronic proximity switches inform the software of the lid's position (fully open, closed or in between). Aluminium is used for the main construction of the 500 mm x 500 mm x 180 mm chambers, with acrylic lids on top allowing light penetration for pasture or crops (van der Weerden *et al*. 2013).

Figure 5.4: Automated chambers developed by AgResearch (New Zealand). Chamber open (left) and closed (right)

In some applications, fans mix the chamber air (Yao *et al*. 2009), and it is commonly assumed in chamber studies that the air space is therefore well-mixed. However, this assumption may not be correct regarding measurements over a deep, dense crop, when N_2O emitted from soil is likely to build up to a much higher concentration at the bottom of the canopy. Using a model, Meyer *et al*. (2001) found that trace gas transport through the canopy is unlikely to introduce errors into the flux estimates, despite a significant concentration gradient. Thus, complete mixing within the air space of the chamber is not always necessary for valid chamber measurements. However, to reduce uncertainty when extensions are fitted, the internal sampling port can be extended vertically to ensure gas is sampled from throughout the chamber.

In high-temperature environments, a slightly tinted acrylic is recommended. This reduces incoming infrared radiation bands with minimal impact on photosynthetically-active radiation bands. Reflective films can also be used, but their impact on heat and photosynthetic activity within the chambers should be assessed. In both examples described here, the chambers are attached by quick release clamps to stainless steel bases, featuring sharp edges for easy insertion approximately 100 mm into the soil. This gives an airtight enclosure with the topsoil. To minimise the memory effect of the chamber on soil properties and plant growth, at least two bases should be located in each treatment replicate, allowing the chambers to be regularly moved.

5.6 Sampling unit

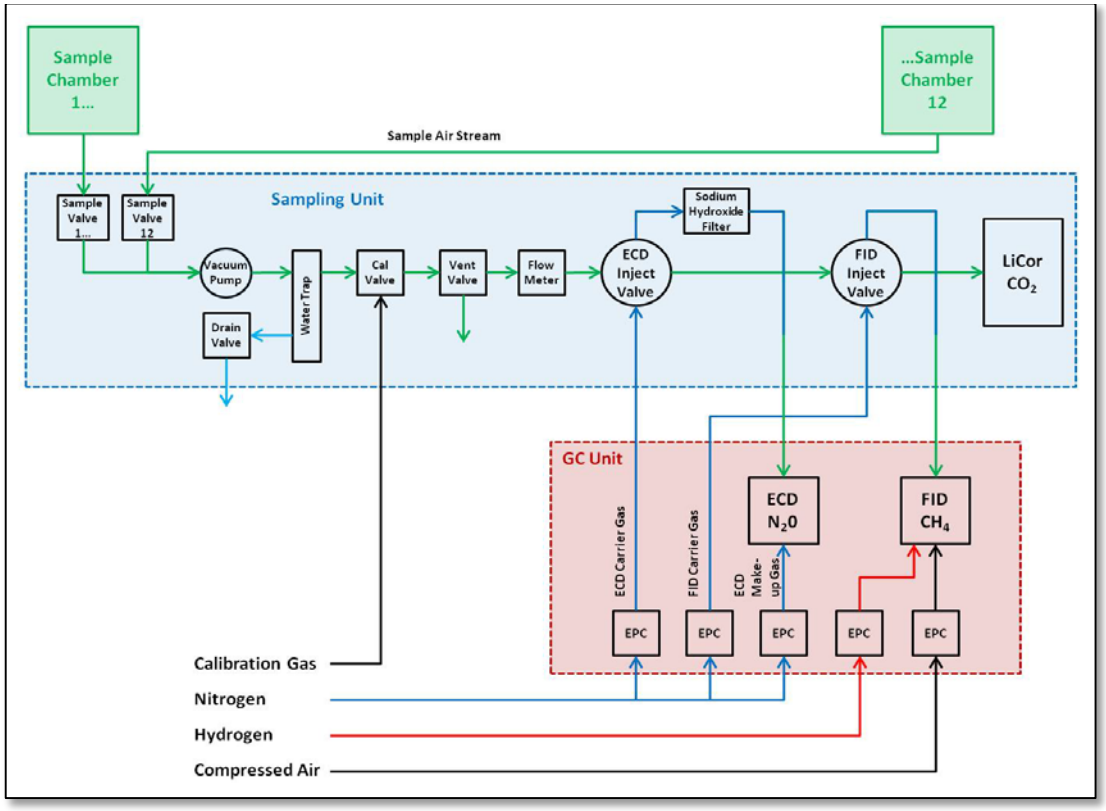
Ordinarily, the Queensland system's sampling unit houses a sample pump, sample valves, calibration gas valve, eight-port GC sample injector valves, sample flow meter, infra-red CO_2 analyser (e.g. LI-820, LI-COR Biosciences), Gas Chromatograph (GC) (e.g. 8610C, SRI Instruments) containing a Flame Ionisation Detector (FID) for CH_4 and an Electron Capture Detector (ECD) for N_2O, pneumatic air regulator and filtering system and a Programme Logic Controller (PLC). The PLC controls all sample and pneumatic air actuators, and receives and processes analogue sensor data. It is connected to a computer which serves as an interface for system control and data storage.

The chambers are connected to the sampling unit by a non-reactive, Teflon-coated sample line, two pneumatic air lines (for opening and closing the lids) and any external temperature and soil moisture sensors required. Each field chamber is connected to the sampling unit by a 1/8" Teflon tube, which is in turn connected to a bank of chamber selection valves (sample valves). When a sample valve opens, a suction pump extracts the sample air from the corresponding chamber. The sample then passes through a water trap to remove any excess moisture. The sample air is then pumped through two, eight-port, two-way Valco (Valco Instruments Co Inc.) injector valves, filling sample loops. The remaining sample air flows to the CO_2 analyser.

After the three-minute sampling time, the injector valves switch, allowing a carrier gas to push the sample out of the sample loops and into the GC separation columns, and to the detectors for analysis (FID for CH_4 and ECD for N_2O). The function of the carrier gas is to 'flush' the 3 mL sample from the sample loops of the Valco injector valves, through the GC separation columns and into the FID and ECD detectors. The carrier gas needs to be an inert gas, providing a stable baseline signal from the detectors. The automated greenhouse gas measurement system uses high-purity nitrogen gas as the carrier gas. There are two independent carrier gas streams: one each for the ECD and FID. Their flows are controlled by electronic pressure controllers positioned in the GC.

A calibration gas is also injected to the GC at regular intervals throughout the sampling cycle via a two-way valve, located before the injector valves. The calibration standard is required for calculating N_2O and CH_4 concentrations from chromatogram peak areas, and for calibrating the CO_2 sensor. For low-emitting systems, typical calibration standard concentrations are: N_2O (0.5 ppm), CH_4 (4 ppm) and CO_2 (800 ppm) (See Chapter 4 for details of the GC calibration, especially noting the non-linearity of ECD detectors.). Where a field scenario emits large amounts of GHG, it is recommended that a higher concentration standard be used, especially for the non-linear ECD detector response. This can be done for short periods at monthly intervals. A schematic diagram of the overall sampling process is provided in Figure 5.5.

Figure 5.5: Sample system schematic, showing the sample air path and carrier gases and calibration gas for the 'Queensland' system as used in Australia's Nitrous Oxide Research Program (Grace *et al.* 2010)



The Valco injector valves send a specific volume of sample air from the automated chambers to the GC detectors for analysis. The standard volume used with this system is 3 mL. Both injector valves are identical, fitted with two 3 mL sample loops each. The ECD and FID injector valves operate in exactly the same way: the sample flows through the ECD injector valve first, followed by the FID valve, and then on to the Li-Cor CO_2 analyser.

A flow meter controls the flow of sample air. The sample flow rate needs to be high enough to ensure the sample air from the chamber reaches the injector valves, and fills the sample loops within the three-minute sampling time. However, the volume of air extracted from the chambers should be minimised, to reduce dilution of the headspace with external air through the chamber vent. A chamber dilution of less than 5% is recommended. This is determined by the volume of the sample removed, divided by the chamber volume (including extensions if fitted).

The minimum sample flow rate is found by dividing the volume of the longest allowable sample line (e.g. 50 m) by the three-minute sampling time, giving a flow rate in mL/min. The recommended flow rate is between 200 and 300 mL/min under normal conditions. The flow rate can, however, be adjusted to the site conditions, taking into consideration the length of sample lines and the use of chamber extensions.

Oxygen as well as any CO_2 and H_2O in the sample air should be removed before the online N_2O analysis by GC can occur, as these gases are detected by the ECD, and may interfere with the accuracy of the N_2O detection. A pre-column with a filter, containing sodium hydroxide coated in silicate (Ascarite), normally does this. The sodium hydroxide will absorb any moisture and CO_2 contained in the sample air.

The AgResearch system includes a mobile caravan housing an Innova 1312 photoacoustic trace gas analyser (TGA; Lumasense Technologies, California), sample valves and controllers, and purpose-designed software using Labview (National Instruments, Texas). The system can be powered by mains supply or by six 125W solar panels and four 6V 420 amp.hr wet cell Trojan batteries, with a backup generator with a 100 amp DC alternator, powered by a 6.5HP petrol engine with 23 litre fuel tank. When the battery voltage drops below 24V, the generator will auto-start and run for one hour, increasing battery voltage to ~28V. All data, including calculated fluxes, are sent via modem to a secure web address, allowing access from any internet connection.

Each chamber has two 20 metre-long, non-reactive Teflon-coated sample lines that connect to a solenoid valve manifold in the caravan. The software communicates with the TGA and solenoid valve controllers to ensure a closed loop is created between a single chamber and the TGA, before switching airlines to the next chamber. The TGA's internal pump, flowing at up to 1.9L/min, circulates air from the chamber headspace into the TGA sample cell, then back to the chamber. N_2O, carbon dioxide (CO_2) and water vapour can be measured every two minutes. Because CO_2 interferes with the TGA's N_2O signal (de Klein *et al*. 1996), a correction factor is established, using a range of mixed CO_2 and N_2O gases of known concentration in N_2. In addition, CO_2 concentration in the air stream is minimised prior to TGA analysis, using a soda lime trap (400 mm length x 10 mm diam.), which is renewed when CO_2 concentrations exceed 1000 ppm CO_2. Water vapour interference is automatically compensated for by the analyser.

To provide a check on calculated fluxes from the automated system, three manual gas samples are periodically collected from chamber headspaces at 25- to 30-minute intervals, following the same method used for manual static chambers, with access to the headspace provided by a rubber septum inserted into the chamber lid. These gas samples, stored in 6 mL vials, are analysed by gas chromatography by the NZ-NCNM, as described in Chapter 4, and calculated fluxes are compared to those produced by the automated system. To date, the comparisons have been favourable (R^2 between 0.93 and 0.98).

Both systems are designed with alarms which activate during certain weather conditions. As previously noted, when the chambers are closed for sampling, they potentially become miniature greenhouses: under high solar radiation, the interior temperature of the chambers can rise excessively. In this case, the Queensland system will interrupt sampling, and open the chamber lids to allow air circulation to lower the temperature, so the vegetation growing inside is not harmed. A rain gauge is also fitted to both systems – the chambers will be opened during rain, or sprinkler irrigation.

The detection limit of the Queensland system is approximately 0.5 g N_2O-N/ha/d (2 µg N_2O-N/m^2/hr) without chamber extensions, and 2.0 g N_2O-N/ha/d (8 µg N_2O-N/m^2/hr) with the 50 mm chamber extension in place (Scheer *et al*. 2012). However, Barton *et al*. (2010) reported lower limits. The detection limit of the AgResearch system is approximately 10.0 g N_2O-N/ha/day (van der Weerden *et al*. 2013), due to the lower sensitivity of the Innova analyser compared to G.C. These limits should be independently determined for each system.

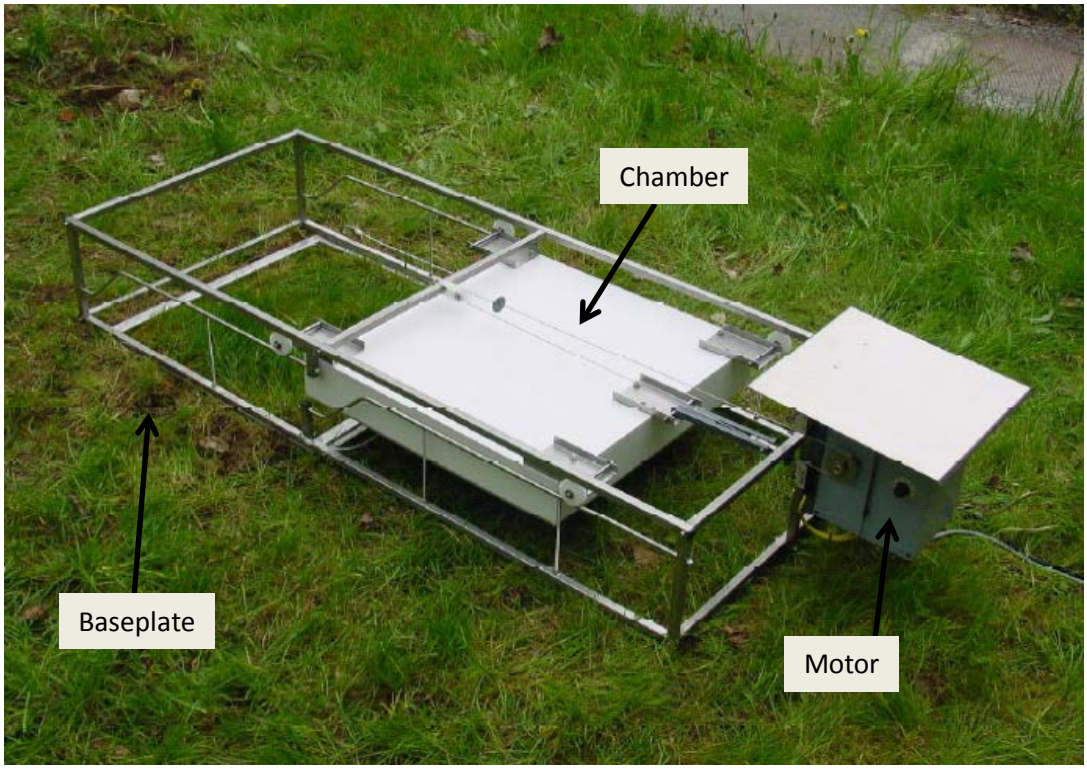
An example of an automated system – in which gas samples are collected in vials which are then returned to the laboratory for subsequent analysis – is provided by a commercially available device that has been developed by Umwelt- und Ingenieurtechnik GmbH (UIT) in Germany (http://www.uit-gmbh.de). The system (illustrated in Figure 5.6 a and b) collects samples from an automatically operated chamber, which opens and closes by the action of an electric motor moving the chamber across a set of guide rails. The chamber closes by being placed on the baseplate and sealed with a silicone tube in the lower rim of the coverbox.

Once the chamber is closed, a hypodermic needle is inserted into pre-evacuated vials, and a membrane pump then flushes the vial with gas sampled from the closed chamber. At the end of the closure period, the chamber is moved away from the baseplate. The system is fully programmable but would, for example, be set to collect three samples over 40 minutes (at 0, 20 and 40 minutes), and between one to four sampling events per day. Samples are stored in airtight glass vials on a sample turntable, and can be transferred directly onto the auto-sampling unit of a GC for N_2O analysis in the laboratory. The system requires a power supply to run the motor that moves the chamber, and powers the vacuum pump. This can be supplied either by a mains supply, or by rechargeable batteries where mains power is unavailable.

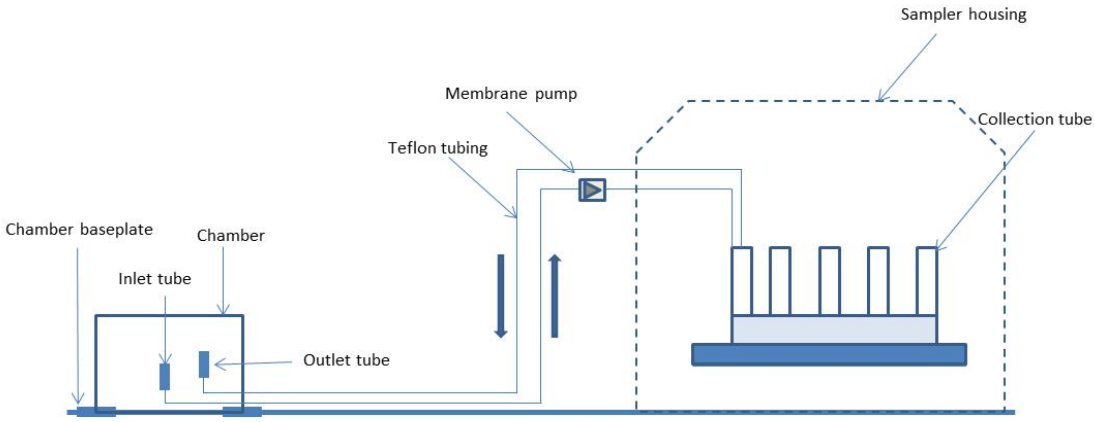
An example of N_2O measured by a UIT auto-sampler compared with that from static chambers is provided in Figure 5.6. These data show that although the auto-sampler has a greater potential to capture temporal variability in fluxes, spatial heterogeneity within the site makes it impossible to determine any statistical difference in the cumulative flux estimated by the two methods. The UIT auto-sampler and similar systems have the advantage of relative simplicity, in that it is used only for the collection and storage of samples. Samples are analysed in controlled laboratory environments, avoiding the need for maintenance of delicate analytical equipment in the field. A network of such samplers is currently being used in the study of N_2O emissions in the UK (Skiba *et al*. 2012). Arnold *et al*. (2010) present a further example of a chamber sampling system developed by the U.S. Dept of Agriculture.

Figure 5.6: (a) The chamber section of the UIT auto-sampler, showing the moveable plastic chamber, rails and electric motor. (b) A diagrammatic sketch of the relationship between the auto-sampler and collection system used by the UIT auto-sampler

(a)



(b)



5.7 Conclusion

Fully automated greenhouse gas measuring systems are now reasonably portable. They can capture highly episodic emissions, and the characteristic diurnality in emissions, by multiple sampling over any 24-hour period. Automated systems offer high temporal resolution data suitable for model calibration, which can be supplemented by a satellite manual sampling network for model validation. However, large discrepancies in emission estimates compared to manual sampling strategies have been quantified.

Where relative differences in emissions associated with different management treatments are used, well-replicated manual chambers remain important tools. While improvements in analytical, sampling and computing technologies have made automated systems more affordable, their utility and uptake depends on country-specific labour costs.

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6 DATA ANALYSIS CONSIDERATIONS

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Summary table

Table 6.1: Summary of recommendations for data analysis

Section/Topic	Objective	Minimum requirements	Evolving issues
Selecting and use of a flux calculation method	Selection and use of most appropriate calculation method for transforming raw chamber headspace concentration data into flux values.	Method should be matched to number of sampling points collected (see Table 6.2, and also Chapter 3 - Chamber Deployment).	Criteria for site-specific selection of best non-linear scheme need to be developed.
Estimation of emissions using non-continuous flux data	Estimate total N_2O emissions occuring over a given time period and given area of field, using flux values from discrete sampling events and small areas.	Daily fluxes can be integrated, using trapezoidal integration. To improve accuracy of cumulative emissions estimates, (i) maximise sampling frequencies and spatial replication given available resources, (ii) repeat experiments over multiple years, and (iii) consider using spatial or temporal gap filling procedures.	Use of automated chamber systems (see Chapter 5) can help minimise temporal uncertainties, but better estimates of spatial variability require a very large number of chambers, or the use of non-chamber (e.g., micro-metereological) methods.
Assessment of minimum detectable flux (MDF)	Determine the minimum flux value that can be measured for a given chamber design and analytical instrument configuration.	Determine random measurement error associated with sampling and analysis of replicate standards of known concentration, and use resulting error rates to determine MDF.	Different flux calculation schemes can differ in their MDF, therefore selection of flux calculation scheme can change MDF (see evolving issues for section 6.1).

Statistical considerations for analysing inherently heterogeneous flux data	Represent central tendency and variance of flux measurements, and compare fluxes among experimental treatments.	If treatments are replicated (at least three blocks), the variability between replicates can be assessed by calculating means of chambers in each replicate; but also the variability within the replicate by assessing the chamber variability.	To assist with comparison of heterogeneous chamber datasets, record the spatial coverage of observations (chamber area times the number of chambers, relative to the plot size: i.e., the plot area covered by the chambers). Advanced techniques are being developed to improve description of non-normal and spatially heterogeneous data-sets and use this to select the best method for mean estimation.
Estimation of emission factor (EF)	Estimate the proportion or percentage of N applied as urine, manure, or fertiliser that is emitted as N_2O.	Inclusion of no-N control treatment, and subtraction of cumulative emissions in control from cumulative emissions in experimental treatment(s) receiving N addition.	Non-linearity of N_2O response to N addition needs to be assessed in different systems (i.e., EFs may vary, depending on rate of N input).

6.1 Selection and use of a flux calculation (FC) method

The first step in any analysis of chamber N_2O data is to calculate the flux from the basic chamber concentration versus time data. It is well documented that selection of a FC scheme can substantially alter the magnitude of flux estimates, as well as the sensitivity to detecting fluxes (Parkin *et al*. 2012). Levy *et al*. (2011) concluded that selection of a FC method was the largest single source of uncertainty in flux estimates from individual chambers.

The various FC schemes differ in their theoretical basis, numerical requirements and potentially, their accuracy and precision. Presently, there is no single clear or perfect choice for the 'best' FC scheme for all applications, and it is not our intention to make a specific recommendation. Our objectives here are instead to summarise the key attributes – and potential limitations – of the most widely used FC schemes from both practical and theoretical perspectives, so that users can make informed decisions for particular applications. We will also make some recommendations on FC scheme selection, based on the number of sampling points collected during each deployment period (DP). This approach is taken because the number of sampling points largely determines the overall suitability of the different schemes.

6.1.1 Basic considerations

Non-steady-state chambers rely on the accumulation of the gas of interest (in our case, N_2O) within an open-bottom chamber placed on the soil surface. The presence of the chamber is likely to affect gas diffusion: in theory, accumulation of N_2O in the chamber immediately suppresses the vertical gradient in N_2O concentration, thereby suppressing the flux below its pre-deployment value (F_0) (Anthony *et al.* 1995).

Chamber placement may also create horizontal gradients in gas concentration, as well as pressure gradients that may further alter the flux (Mathias *et al*. 1978; Pedersen *et al*. 2010). The net result of at least the first two of these effects is that they lead to non-linearity in the relationship between chamber concentration (C) and time (t) after deployment, such that the maximum value of the slope (dC/dt) occurs immediately after chamber placement and decreases over time. This alteration in the slope complicates the estimation of F_0, and selection of a FC scheme. While F_0 will be best represented by the slope value occurring immediately after chamber placement, determining the initial value of dC/dt can be problematic in practice.

Table 6.2: Summary of key advantages, disadvantages and recommendations for selection of Flux Calculation (FC) Scheme

Scheme	Advantages	Disadvantages	Recommendations
Conventional FC schemes			
LR: **(Linear regression)**	Least sensitive to measurement error (most precise) of all methods. Least-biased method for convex-upward curvature. Computationally simple.	Empirical, with no basis in diffusion-theory. Most biased method for convex-downward curvature.	Recommmended option with: three sampling points, or; > 3 sampling points, and convex-upward curvature is observed.
HM **(Hutchinson and Mosier)**	Based on quasi steady-state diffusion theory. Least-biased conventional scheme for convex-downward curvature.	Restricted to three equally-spaced time points. More sensitive to measurement error (less precise) than LR and QR.	Not recommended, because of high imprecision and availability of improved non-linear methods.
QR **(Quadratic regression)**	Not limited to three equally-spaced sampling points. More precise than HM method. Less biased than LR for convex-downward curvature.	Empirical, with no basis in diffusion-theory. More biased for convex-downward curvature than other non-linear methods.	Recommended option with: ≥ 4 sampling points.
Advanced FC schemes			
NDFE **(Non-steady state diffusive flux estimator)**	Based on non-steady state, one-dimensional diffusion theory, with clearly defined physical assumptions. Provides 'perfect' calculation of flux at time zero, when all assumptions are held and with no measurement error.	Highly sensitive to violation of underlying assumptions. Can deliver more than one flux value for a given data set and/or unexpectedly high flux values. Not easily adapted to spreadsheets, nor efficient for handling large data sets.	Recommended option with: ≥ 4 sampling points.

HMR **HMR method**	Based on same theory as HM method, but with additional consideration of lateral (two-dimensional) gas transport beneath chambers. Available as part of software package that provides confidence intervals for estimated flux values.	More sensitive to random measurement error (less precise) than LR and QR, especially at lower flux values.	Recommended option with: ≥4 sampling points.
CBC **(chamber bias correction method)**	Same theoretical basis as NDFE method. Delivers a single flux value, avoids unexpectedly high flux values given by NDFE and less sensitive to violation of assumptions than NDFE. Can be combined with QR or LR methods.	Requires additional soil data, which may introduce error. Requires multiple calculations (but can be done in spreadsheet format).	Recommended option when accurate soil bulk density and water content data are available, with: ≥ 3 sampling points when combined with LR or, ≥ 4 sampling points combined with LR or QR.

6.1.2 Conventional FC schemes

We refer to linear regression (LR), the method of Hutchinson and Mosier (1981) (HM), and quadratic regression (QR) (Wagner *et al*. 1997), as 'conventional' methods, because they have traditionally been the most commonly used across the world, and also because all three methods allow for direct calculation of flux using the equation:

$$F = H\frac{dC}{dt} \quad (1)$$

where F is flux (with units[4] of M L^{-2} T^{-1}), *H* (L^3 gas L^{-2} soil) is the ratio of the internal chamber volume to surface area in contact with the soil – commonly referred to as chamber 'height' (with units simplified to L) – *C* is the N_2O concentration in the chamber (M L^{-3} gas), and *t* is time (T). The designation *dC/dt* is used to represent the time rate of change in *C* (M L^{-3} gas T^{-1}). The LR, QR, and HM methods each aim to determine *dC/dt* for use in Eq. [1].

6.1.2.1 Linear regression

About 75% of studies reporting NSS-based N_2O fluxes published between 2005 and 2007 used LR as the FC scheme (Rochette & Eriksen-Hamel 2008). The LR approach

[4] Unit dimensions are indicated by M for mass, L for length, and T for time. Where appropriate, dimensions are also specified with respect to the quantity described by the unit: i.e., soil or gas.

simply uses the slope obtained from least-squares linear regression of *C* versus *t* to estimate *dC/dt* for use in Eq. [1]. Obviously, applying LR to inherently non-linear data, as described above, will in theory tend to underestimate F_0, and this has been shown in several studies (e.g. Matthias *et al*. 1978). While this is universally recognised, LR is nevertheless widely used because of its practical advantages. It is computationally simple, and applicable to low numbers of chamber observations (e.g. *n*=2). However, while using two time points per chamber deployment may be attractive logistically, it does not allow for any evaluation of non-linearity, nor the statistical confidence of the estimate.

Some researchers have justified the use of LR and/or two sampling points, based on preliminary measurements showing a high degree of linearity in chamber data for a particular site. However, diffusion theory predicts that: (i) even relatively small deviations from linearity can result in substantially biased LR-based flux estimates; and (ii) the extent of non-linearity in chamber data can vary considerably among measurements, depending on soil physical properties (e.g. water content), which can range widely over time and space (Livingston *et al*. 2006; Venterea and Baker 2008).

For example, Conen and Smith (2000) used numerical modelling to show that when LR was applied to theoretical chamber data exhibiting r^2 values greater than 0.997, F_0 was underestimated by more than 25%, even at a relatively low value of soil air-filled porosity (i.e., 20%). Venterea and Parkin (2012) showed how increasing air-filled porosity leads – in theory – to increased non-linearity in chamber data and correspondingly increased underestimation of F_0, due to increasing accumulation of gas within the soil pores instead of the chamber. Conen and Smith (2000) refer to this phenomenon as N_2O "storage" within the soil profile.

Venterea and Parkin (2012) and Venterea and Baker (2008) demonstrated that such soil property effects on flux underestimation imply that LR (and potentially other FC schemes) will be more or less accurate at different times and/or in different places during a field experiment, thereby leading to biases that could confound the results. Nevertheless, compared with the QR and HM schemes, LR-based estimates are least sensitive to random variations in chamber N_2O concentrations resulting from sampling techniques and performance of analytical instruments: in other words, from variations arising from 'measurement error' (Venterea *et al*. 2009). Similarly, LR has been shown to have the lowest method detection limit, compared with other schemes (Parkin *et al*. 2012).

In this sense, LR can be said to have greater *precision* compared with other schemes, while at the same time having the greatest expected *bias*. Furthermore, LR's precision relative to other FC methods is expected to increase as the number of sampling points (n) collected per DP decreases (Venterea *et al.* 2009). This fact, combined with the lack of statistical robustness of non-linear FC methods when $n < 4$ (see sections below), leads us to recommend that LR be used when $n = 3$.

In addition, under certain circumstances, precision might be considered of equal or perhaps greater importance than bias. For example, Venterea *et al*. (2009) showed that LR-based flux estimates can be more statistically robust for detecting differences

in fluxes among experimental treatments, by reducing the additional variance contributed by measurement error.

The advantage of LR in this regard will depend on the magnitude of the flux in relation to measurement error, and to other factors which may be difficult to predict (Venterea *et al*. 2009). One option is to calculate fluxes using both LR and a non-linear scheme, then determine if means comparisons or statistical relationships using LR-based flux estimates are more robust. Of course, in this case, it must be kept in mind that the LR-based estimates will more greatly underestimate F_0 than a non-linear scheme.

Another situation where LR may be the only reasonable option is when a chosen non-linear scheme 'fails' when applied to a particular set of chamber data. All other FC schemes essentially assume that chamber data will have decreasing slope over time. In practice, measurement error and/or other factors (e.g. temperature or pressure variations) may result in data that display near-perfect linearity or curvature that is 'opposite' to the expected pattern (i.e., increasing slope over time). In the latter case, non-linear FC schemes tend to produce a flux estimate less than that produced by LR, which is an unreasonable outcome.

Thus, when using methods other than LR, it is advisable to evaluate each individual data set for method 'failure', as discussed below. In these cases, use LR, or perhaps remove any clearly anomalous data points responsible for the method failure.

6.1.2.2 The HM method

The non-linear FC scheme, first proposed by Hutchinson and Mosier (1981), is very commonly used in N_2O work. However, the theoretical basis and underlying assumptions of the HM model may not be as widely understood. The assumptions are that: (i) the N_2O gas concentration at some depth *d* in the soil is a constant (C_d) during the chamber deployment period; (ii) the physical properties (e.g., water content, bulk density) that control soil-gas diffusion are uniform in the soil layer above the depth *d*, and (iii) the flux of gas into the chamber is controlled by one-dimensional (1D) vertical diffusion, proportional to a linear soil-gas concentration gradient (*dC/dt*) between *d* and the soil surface.

With these assumptions, the rate of change in chamber N_2O concentration (*C*) can be described by a simple ordinary differential equation given by:

$$\frac{dC}{dt} = k(C_d - C) \qquad (2)$$

Where $k = \frac{D}{Hd}$, and *D* is the soil-gas diffusion coefficient (L^3 gas L^{-1} soil T^{-1}) in the soil layer above *d*. It is mathematically straightforward to find a general solution to Eq. [2] that could be used to estimate the flux at time zero, but this would result in a FC scheme requiring non-linear regression, therefore preventing the direct use of Eq. [1]. To avoid this, Hutchinson and Mosier (1981) limited their application to the case where the chamber is sampled immediately upon deployment, and then again at two equally-spaced time intervals. In this case, *dC/dt* at *t*=0 can be determined from:

$$\frac{dC}{dt} = \frac{(C_1 - C_0)^2}{\Delta t(2C_1 - C_2 - C_0)} \ln(\alpha) \qquad (3)$$

where C_0, C_1, and C_2 are the chamber N_2O gas concentrations measured immediately after chamber deployment, after the first interval, and after the second interval, respectively, Δt is the time interval between each sample, and $\alpha = \frac{(C_1 - C_0)}{(C_2 - C_1)}$. In this case, F can be calculated directly from Eqs. [1] and [3].

In addition to being restricted to the case of three equally-spaced time points, Eq. [3] will fail when $\alpha=1$ ($F = 0$) and when $\alpha \leq 0$ ($\ln(\alpha)$ is not defined). Also, when $0 \leq \alpha \leq 1$, unexpected curvature will occur as discussed above. Thus, combining these three cases, reasonable model failure criteria for the HM method would be to exclude all cases where $\alpha \leq 1$, in which cases applying LR instead may be more reasonable (Venterea *et al*. 2009).

The main advantage of the HM method is that it has some degree of theoretical basis: it is computationally straightforward, and allows for explicit use of Eq. [1]. On the other hand: (i) compared with LR and QR, the HM method has been shown to be most sensitive to measurement error, and therefore less precise than these other methods; (ii) HM cannot be used with > 3 sampling points, unless an averaging procedure is used – for example, by using four equally-spaced time points and using the average of the middle two time points as the second point – and (iii) HM cannot generate statistical data (e.g., confidence intervals, r^2 values). For these reasons, the HM method is not recommended.

6.1.2.3 Quadratic regression

The quadratic regression (QR) method proposed by Wagner *et al*. (1997) assumes that chamber gas concentration will change as a function of time, according to:

$$C(t) = at^2 + bt + c \qquad (4)$$

where a, b, and c are regression coefficients. Because the first derivative of Eq. [4] at t=0 is equal to b, the flux at time zero (F_0) can be estimated by substitution of b for dC/dt in Eq. [1]. Like LR, QR is empirical, with no physical basis. The QR method can be applied without necessarily using non-linear regression; for example, the multiple regression (LINEST) function in Microsoft Excel can be applied in spreadsheets by treating t and t^2 as separate independent variables.

The QR method can be used with more than three sampling points, and – in contrast to the original HM method – with any (e.g. non-uniform) sampling interval. Because Eq. [4] contains three regression coefficients, more than three sampling points are recommended when using QR. When more than three sampling points are used, the LINEST function can be used to return model statistics, including R^2 and the standard error of the estimate of b. In contrast, the original HM model allows for only three equidistant sampling points; therefore model statistics cannot be determined (limitations in number and distribution of samples are overcome in the HMR model, see section 6.1.3.3).

Because Eq. [4] can be fitted to data displaying a wide range of non-linear patterns, it is recommended that model failure criteria be used when applying the QR method. Evaluation of model failure can be facilitated by using the value of the second

derivative of Eq. [4], which is equal to *2a*. Unexpected data curvature will occur whenever *a* and *b* have the same sign, or in other words, whenever *ab*> 0. QR is more flexible in terms of sampling regime, and less sensitive to measurement error, compared with HM (Venterea *et al*. 2009). Theoretical analysis has indicated that QR produces more accurate flux estimates than LR, but less accurate than HM in the absence of measurement error (Livingston *et al*. 2006; Venterea *et al*. 2009).

6.1.3 Advanced FC schemes

We apply the term 'advanced' to FC schemes which have a more rigorous or extended theoretical basis than conventional schemes, and which require additional numerical computation beyond direct calculation using Eq. [1]. Included in this category are the NDFE (Livingston *et al.* 2006), CBC (Venterea 2010), and the extended HM/HMR methods (Pedersen *et al*. 2010). Each of these schemes has its advantages and disadvantages, and currently, neither can be recommended as better overall. We do, however, recommend that when ≥ four points are sampled, a non-linear scheme be used: the recommended options therefore include LR with CBC, QR with or without CBC, NDFE alone, or HMR alone. The discussion below is provided so that users can make informed decisions about FC scheme selection.

6.1.3.1 The NDFE method

The non-steady state diffusive flux estimator (NDFE) scheme developed by Livingston *et al*. (2006) is derived from a more rigorous theoretical basis than any other scheme. The major advance of the NDFE method is that it derived a useful solution to a partial differential equation (PDE) describing soil-gas production, diffusion, and accumulation in a chamber under transient (non-steady state) conditions. Furthermore, it is not confined to N_2O production occurring in a specific soil layer, or to diffusion driven by linear concentration gradients. A precise analytical solution to the PDE was obtained by Livingston *et al*. (2006), describing the chamber gas concentration (*C*) as a function of time (*t*) as follows:

$$C = C_0 + F_0 \frac{\tau}{H}\left[\frac{2}{\sqrt{\pi}}\sqrt{t/\tau} + \exp\left(\frac{t}{\tau}\right)\mathrm{erfc}\left(\sqrt{t/\tau}\right) - 1\right] \quad (5)$$

Livingston *et al*. (2006) also published software (available at http://arsagsoftware.ars.usda.gov) which performs non-linear regression analysis and returns a value for F_0. Since the model (Eq. [5]) has a total of three regression parameters (F_0, C_o, and τ), a minimum of four sampling points is recommended, so as to obtain statistically feasible estimates.

The NDFE method is appealing, because it provides a theoretical basis for calculating F_0, but it has some practical and theoretical limitations. The regression solver is not easily adapted to spreadsheets, nor efficient for handling large data sets. Also, different runs of the solver will frequently return different values of F_0 for the same set of chamber data, and in some cases, produce F_0 values much greater than expected, or determined using other methods (Kutzbach *et al*. 2007; Venterea 2010). In these cases, it may not be clear which F_0 values are 'true', and which values result from violation of one or more of the assumptions underlying Eq. [5].

One of these assumptions is that the soil is vertically uniform, with regard to water content and bulk density. Venterea and Baker (2008) showed that the NDFE can underestimate – and in some cases overestimate – F_0 when applied to soil profiles having realistically non-uniform physical properties. Another assumption behind Eq. [5] is that chamber placement does not cause gas to diffuse horizontally beneath the chamber, which would further alter the curvature of the *C* versus *t* data. In other words, the method assumes only 1D diffusion, and therefore predicts in principle that chamber gas concentration will increase *ad infinitum*.

The validity of the assumption of no horizontal diffusion depends on the insertion depth of the chamber base walls into the soil, combined with the soil air-filled porosity, and the duration of chamber deployment. Hutchinson and Livingston (2001; 2002) provided criteria for determining the minimum insertion depth required to minimise this effect.

Livingston *et al*. (2006) numerically investigated the sensitivity of the NDFE model to chamber insertion depth, and found that the use of insertion depths less than those recommended by Hutchinson and Livingston (2001; 2002) resulted in NDFE overestimating F_0. Kutzbach *et al*. (2007) provided some empirical support for the potential importance of horizontal diffusion effects on NDFE-based flux estimates, and its inadequacy under some circumstances, such as shallow chamber insertion depths in porous soils. The extended HM model (section 6.1.3.3) attempts to account for additional non-linear curvature due to horizontal diffusion (Pedersen *et al*. 2010).

6.1.3.2 The CBC method

The chamber bias correction (CBC) method developed by Venterea (2010) utilises the same fundamental theory as Livingston *et al*. (2006), but applies it in a way that avoids non-linear regression. The CBC method is applied by first determining the flux using a conventional FC scheme (LR, HM, or QR). The initial flux estimate is then multiplied by a theoretically-based correction factor, which is calculated from soil physical properties (bulk density, water content, clay content, and temperature), chamber height (*H*) and total chamber deployment period (*DP*).

The CBC method utilises the fact that the τ term in Eq. [5] has physical meaning related to soil physical properties and *H*, and that the error of the initial flux estimate is predictably related to the quantity $\ln\left(\frac{\tau}{DP}\right)$, Venterea (2010) describes the theoretical basis and mechanics for calculation of correction factors. An example spreadsheet is at http://www.ars.usda.gov/pandp/people/people.htm?personid=31831. Advantages of the CBC method are that it preserves the theoretical basis of the NDFE method, but overcomes some of its limitations. For example, it attempts to overcome the assumption of the NDFE method that water content and bulk density are vertically uniform by using soil physical properties averaged over the upper 10 cm of the soil profile. The CBC method avoids the need for a non-linear regression solver, and therefore delivers a single flux value, calculated using a conventional spreadsheet. It avoids generation of extraneously high flux estimates that are sometimes observed with the NDFE method (Venterea 2010; 2013).

On the other hand, the method requires additional soil property data. While these data are commonly available in many studies because of their influence over N_2O production, these additional measurements necessarily introduce additional sources of potential error. The sensitivity of CBC-based flux estimates to errors in soil property measurements has been recently quantified (Venterea and Parkin, manuscript in preparation).

6.1.3.3 The extended HM model and the HMR method

Pedersen *et al*. (2010) developed the HMR method, which builds on the original method of Hutchinson and Mosier (1981) but with expanded applicability. It has seen increasing application in some studies (e.g. Petersen *et al*. 2012). The HMR method is actually a comprehensive flux-calculation software available as an add-on package to be used with the R statistical programme (available at http://cran.opensourceresources.org/). The HMR method includes within it a FC scheme that expands the theoretical basis of the HM model to account for lateral (2D) gas diffusion induced by chamber placement and/or gas leaks from an imperfectly sealed chamber. This is accomplished by modifying the governing equation initially given by Eq. [2] as follows:

$$\frac{dC}{dt} = k(C_d - C) - \gamma(C - C_0) \quad (6)$$

where the term $\gamma(C - C_o)$ accounts for lateral diffusion and chamber leaks. Eq. [6] can be re-arranged in the form of Eq. [2] with different values of C_d and k, but *the same* initial flux, which means that the flux estimate is independent of lateral diffusion and chamber leaks, as modelled by Eq. [6]. HMR can fit the HM model by non-linear regression to concentration measurements from three or more sampling time-points and arbitrary sampling intervals.

Further, HMR uses a one-parameter criterion which facilitates the search for the optimal fit: the HMR estimation procedure restricts the parameter space to ensure that estimated values are valid HM model parameters. The HM model (Eq. [2]) has the linear model (LR) and the constant model (no flux) as limiting cases (LR: $k \rightarrow 0$; No flux: $k \rightarrow \infty$). Therefore, when HMR detects that the criterion function is ever improving for k, approaching either zero or infinity, it recommends data to be analysed by LR, or no analysis, respectively. HMR leaves the choice of analysis to the user, and provides diagnostic plots to support a qualified decision.

For all supported analyses, HMR provides p-values 95% confidence intervals for the estimated flux, based on standard asymptotic statistical theory. The principles of the HMR estimation and classification procedure could also be applied to the NDFE model, which also has the linear and the constant model as limiting cases (LR: $\tau \rightarrow \infty$; No flux: $\tau \rightarrow 0$). As mentioned above, some studies have shown that, in practice, the NDFE model often does not fit measured chamber concentrations well, possibly due to violations of the NDFE assumption of no horizontal gas transport or other assumptions.

Analysing data with low signal-to-noise ratio is particularly challenging with non-linear FC schemes. There is always a risk that chamber concentrations by chance, even at

sites with no flux, will follow a clear non-linear pattern, which may fool the HMR procedure to erroneously estimate a large and seemingly statistically significant flux. The variation of chamber measurements must be evaluated against the site-specific natural variation of the trace gas concentration (e.g., derived from repeated pre-deployment sampling), but this is not presently part of the HMR method.

6.1.4 Criteria for selecting FC scheme for particular applications

Which is the best FC method? As described above, several criteria must be considered when selecting an analysis technique to apply to a given data set. Several 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; Venterea, 2013).

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 (see Chapter 4, section 4.4 - 4.7). 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, the variance of the flux estimation method should also be considered, as well as its bias.

A statistical analysis by Venterea *et al*. (2009) demonstrated clear trade-offs 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. 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. 7) (DeGroot 1986):

$$\text{MSE} = \text{Variance} + \text{Bias}^2 \qquad (7)$$

Parkin and Venterea (manuscript in preparation) investigated these issues further, using Monte Carlo simulation to evaluate the bias, variance, and MSE of linear regression, the HM method, and the Quad method when applied to data sets of three or four 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_2O chamber data, using the method described by Venterea *et al*. (2009). This analysis was applied over a range of analytical precisions (1% to 6%), and showed there is no 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: i) the magnitude of the underlying flux; ii) the degree of data curvi-linearity and iii) the analytical precision. The reader is referred to Parkin and Venterea (2010) for preliminary results of this analysis. Additional analysis is under way (Parkin and Venterea, manuscript in preparation). It is quite possible that analysis of N_2O flux

results from complex environments – where fluxes may range over several orders of magnitude and display different types and degrees of non-linear curvature – will require a combination of FC methods to obtain the best overall precision and minimum bias. The HMR software (section 6.1.3.3) enables the analyst to choose between LR and a non-linear model (or zero flux) for each individual data set, based on scatter plots. This approach could be extended by more stringent criteria to guide the decision on flux calculation method.

6.2 Estimation of cumulative emissions using non-continuous flux data

Accurately determining N_2O fluxes from agricultural soils is a major challenge, due to the large spatial and temporal variability of the microbial processes that generate them, and their interaction with environmental variables. Long-term studies are recommended, as fluxes vary from year to year (Velthof and Oenema 1995): unusual weather in one year will affect subsequent emissions that year, and thereafter.

6.2.1 Accounting for spatial variability

The spatial variability in N_2O emissions (as discussed in Chapter 3) means that large coefficients of variation are often encountered in flux data derived from static chamber measurements: e.g., 50-100% for CH_4 (Whalen and Reeburgh 1988); 13-57% (Yamulki *et al*. 1995) and 31-168% for N_2O (Matthias *et al.*1978). Calculation of mean fluxes from a replicated experiment must therefore give a representative value of the spatial variability of the plot in question. This spatial variability has been considered log-normal at all scales (Oenema *et al*. 1997), although normal distributions have also been reported, in which case arithmetic means are used (Petersen 1999).

It has been suggested that the type of distribution can change at different times of the year (Tiedje *et al*. 1989). Normal distribution would be expected when the soil is wetter and more homogeneous. In the summer, when the soil is dry, hot spots are expected, producing a log-normal behaviour (Parkin 1987; Tiedje *et al*. 1989). A third type of distribution has been reported, in clusters, which shows two or more groups of data (see Chapter 3, section on Strategic Sampling). In this case, a mean per cluster is calculated, and these means are then averaged to give the plot mean. Cardenas *et al*. (2010) observed that the mean of the cluster means was biased by large values when these were a minority in the data set and noted that the bias could have been due to different numbers of data points in each cluster.

Another suggested method is the Kriging technique, in which gaps in data in a field (spatial gaps, areas of the field with no measurements) are filled in, but it relies on spatial autocorrelation between measured fluxes (Folorunso & Rolston 1984). It is however, common to have only few chambers (fewer than 10) to measure fluxes from a particular treatment at field scale, restricting the possibility of attributing the relevant distribution (Velthof & Oenema 1995). In this case, normal distribution is usually assumed and arithmetic means determined (Cardenas *et al*. 2010).

6.2.2 Accounting for temporal variability

As discussed in Chapter 5, the more frequently measurements are made, the more accurate the integrated seasonal/yearly cumulative flux estimate will be (Smith & Doobie 2001; Parkin 2008). When estimating daily and cumulative fluxes, certain components of temporal variability must be considered, including diurnal variations, and variations from perturbation, such as tillage, fertility, irrigation, rainfall and thawing. To account for diurnal variability, it is recommended that fluxes are measured at times of the day that more closely correspond to the daily average temperature (mid-morning, early evening). Q_{10} temperature correction may be used to adjust daily flux 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 soil temperature (depth) 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. Biological reaction rates increase exponentially with temperature between 15 – 35°C, and Q_{10} values found in the literature range between 1.6 for conditions conducive to nitrification (Smith *et al*. 1998), to 15 in heavy soils under wet conditions conducive to denitrification (Dobbie *et al*. 1999; Smith *et al*. 1998).

Temperature also affects the solubility of gases in water, as well as their rates of diffusion in the soil profile, affecting N_2O as well as O_2 diffusion. These in turn affect anaerobicity, suggesting a complex effect of temperature on fluxes. The appropriate Q_{10} factor, then, must be carefully determined when using a temperature correction.

Frequent sampling is recommended to account for temporal variation caused by perturbation, both before and after the events (Chapter 3). To calculate cumulative fluxes, the daily fluxes can then be integrated, using the trapezoidal integration method. However, this method could overestimate fluxes, especially if measurements are carried out more intensively around events (fertiliser application, rainfall) or if measurements are infrequent, especially around the time of larger fluxes.

Therefore, there may be a need to fill in the gaps when there are no measurements taken. This could be done by extrapolating the last pre-perturbation flux measurement over time, until just before the perturbation. Emissions between events (background fluxes) can also be used to calculate mean daily background fluxes, then extrapolated to the year by multiplying by the number of days not affected by events. However, this can underestimate emissions, as changes in soil mineral N (especially when organic carbon is high, or when crop residues are incorporated) could provide the N necessary for the production of N_2O at those times when emissions are not expected to be great (Dobbie and Smith 2001; Webster and Goulding 2006). Empirical or process-based models can also be used to estimate fluxes on those times and locations where measurements were not carried out.

6.3 Assessment of minimum detectable flux (MDF)

Past efforts to assess the minimum detection limits of soil gas emissions have focused on determining goodness-of-fit of regression procedures. For fluxes determined by linear regression, a t-test of the slope of the regression line can be used to assess if the flux is significantly different from zero (Livingston & Hutchinson 1995; Rochette *et al*. 2004). Since standard errors of the model parameters obtained in the Quad and HMR methods can also be calculated, a t-test of significance can be applied to determine the significance of fluxes derived by these methods.

The HM flux procedure does not allow for calculation of an associated standard error directly. However, the stochastic application of the HM procedure developed by Pedersen *et al*. (2001) does provide flux estimates with associated confidence limits, enabling the determination of regression significance. Typically, goodness-of-fit tests are applied at an α level of 0.05. However, in computations of trace gas fluxes with three or four data points, degrees of freedom will be small (degrees of freedom = number of time points, minus number of model parameters). When the number of degrees of freedom is small, the power to detect significance is low, thus the type II[5] error rate will be high. In addition, whereas goodness-of-fit tests can determine whether a given flux is significantly different from zero, they do not provide an indication of the magnitude of the minimum detectable flux.

Using Monte Carlo sampling, Parkin *et al*. (2012) developed a method to determine the minimum detection limits for several different regression models when three or four data points are available. This method allows the calculation of the flux minimum detection limit if the chamber deployment time (DT) and sampling/analytical precision (coefficient of variation) are known (see Appendix 3 for an example of this calculation).

6.4 Statistical considerations for analysing inherently heterogeneous flux data

6.4.1 Assessment of normality and transformation

The high variability of N_2O emissions often manifests as positively skewed distributions. These in turn arise because many environmental variables cannot take on negative values, and are therefore constrained by zero. Before applying any standard analysis of variance procedures, several assumptions must be established concerning the underlying error structure of the data. Among these is the assumption of normality. The effects of violations of the assumption of normality on the efficacy of parametric statistical tests, such as the t-test, have long been known (Hey, 1938; Cochran, 1947).

Non-normality will influence the ability of a statistical test to perform at the stated a-level – an effect Cochran (1947) refers to as the validity of the test. Non-normality also affects the power of a statistical test to detect differences when real differences in the data actually exist. Two common procedures have been recommended for when data

[5] Failure to reject false null hypothesis

are not normally distributed: (i) transform for normality, or (ii) apply nonparametric statistical methods (Snedecor and Cochran 1967).

These two approaches, though, have consequences for the inference base – specifically with regard to the estimand – which are not typically considered. This discussion will focus on log-normally distributed data, and present information on (i) optimal methods for computing the mean and variance for a log-normally distributed variable, and (ii) guidelines for hypothesis testing.

6.4.2 Estimating the mean and variance of log-normally distributed data

In most environmental studies, it is impossible to sample the entire population of the variable of interest. Thus, we are forced to estimate the parameters of the underlying population – such as the mean and variance – from sample data. Estimating the mean and variance for normally distributed data is straightforward. But sample data is often positively skewed, and is better approximated by the two-parameter log-normal distribution. When log-normality exists, statistical methods of analysing Gaussian data are not ideal: there are better techniques for estimating the population mean, median, and variance from sample data (Parkin and Robinson 1992; Parkin *et al*. 1988).

These alternatives yield unbiased parameter estimates, and have minimum variance. In addition, exact methods for computing confidence limits of the mean and median are known (Parkin *et al*. 1990). Three methods have typically been applied to estimate the mean and variance of log-normally distributed data. These are the method of moments (MM), the maximum likelihood method (ML) and the uniformly minimum variance unbiased estimator (UMVUE) method.

6.4.2.1 Method of Moments estimators (MM)

Method of Moments (MM) estimators are computed according to the standard methods found in common statistical texts (the mean is the arithmetic average of the sample values, and the variance is the average squared deviation from the mean):

$$m = \frac{1}{n}\sum_{i=1}^{n} x_i \tag{8}$$

$$s^2 = \frac{1}{(n-1)}\sum_{i=1}^{n}(x_i - m)^2 \tag{9}$$

where x_i = the untransformed ith observation, n = the number of observations, m = the estimate of the population mean, and s^2 = the estimate of the population variance.

The MM estimators are unbiased, irrespective of the underlying distribution. However, they have higher associated variance than the UMVU estimators when applied to log-normal data, and so are less efficient.

6.4.2.2 Maximum Likelihood estimators (ML)

Maximum Likelihood (ML) estimators employ the use of log-transformed sample data and compute the mean according to the asymptotic formulae shown below.

$$m = e^{(\hat{\mu}+\frac{\sigma^2}{2})} \tag{10}$$

$$\sigma^2 = \frac{1}{(n-1)}\sum_{i=1}^{n}(\ln(x_i) - \hat{\mu})^2 \quad (11)$$

where:

$$\hat{\mu} = \frac{i}{n}\sum_{i=1}^{n}\ln(x_i) \quad (12)$$

and:

$$\sigma^2 = \frac{1}{(n-1)}\sum_{i=1}^{n}(\ln(x_i) - \hat{\mu})^2 \quad (13)$$

In some literature, these ML estimators have been recommended when the sample data conforms to a log-normal distribution. However, it has been shown that these estimators are biased, and inefficient for small sample sizes (n<1000). They are therefore not recommended (Parkin *et al*. 1988).

6.4.2.3 Uniformly Minimum Variance Unbiased estimators (UMVU*)*

The Uniformly Minimum Variance Unbiased Estimators (UMVUE) were developed independently by Finney (1941) and Sichel (1952), and have been typically applied to the analysis of geological data (Krige 1981; Koch & Link 1970). Estimators of the population mean (m) and variance (s^2) are given by Eqs. [14] and [15], respectively.

$$m = e^{\hat{\mu}}\varphi(\sigma^2/2) \quad (14)$$

$$s^2 = e^{2\hat{\mu}}\left\{\varphi(2\sigma^2) - \varphi\left[\frac{(n-2)}{(n-1)}\sigma^2\right]\right\} \quad (15)$$

where φ = the power series, described in Eq. 16:

$$\varphi(t) = 1 + \frac{t(n-1)}{n} + \frac{t^2(n-1)^3}{n^2(n+1)2!} + \frac{t^3(n-1)^5}{n^3(n+1)(n+3)3!} + \frac{t^4(n-1)^7}{n^4(n+1)(n+3)(n+5)4!} + \ldots \quad (16)$$

Thus, to calculate the UMVU estimate of the sample mean (Eq. 14), the term ($\sigma^2/2$) would be substituted for 't' in the power series (Eq. 16). To estimate the UMVU variance (Eq. 15), the power series would have to be solved twice; once with the term ($2\sigma^2$) substituted for 't' in Eq. 16 and once with the term $\left[\frac{(n-2)}{(n-1)}\sigma^2\right]$ substituted for 't'. It is recommended that the power series (φ) be evaluated until the final term accounts for <1% of the sum of the preceding terms. This usually requires the calculation of six to ten terms.

The application of these three techniques (MM, ML and UMVU) to estimate the mean and variance depends on sample size, and the variability of the underlying population (as indicated by the sample coefficient of variation). Recommendations for application of these techniques are given in Table 6.3. Details from evaluations of these methods are presented by Parkin *et al*. (1988).

Table 6.3: Summary of recommended methods for estimating the mean and variance of log-normally distributed populations for three sample coefficients of variation (CV) by three sampling intensity ranges. When more than one method is recommended, the methods are presented in order of most, to least, preferable. MM: method of moments, ML: the maximum likelihood method, UMVUE: the uniformly minimum variance unbiased estimator method

Sample CV	Sample Size (n)	Recommended Method	
		Mean	Variance
50 %	4 – 20	MM, UMVU	MM, UMVU
	20 – 40	MM, UMVU	UMVU
	40 – 100	MM, UMVU	UMVU, ML
100%	4 – 20	UMVU, MM	UMVU
	20 – 40	UMVU, MM	UMVU
	40 – 100	UMVU, MM	UMVU
200 %	4 – 20	UMVU	UMVU
	20 – 40	UMVU	UMVU
	40 – 100	UMVU, ML	UMVU

6.4.2.4 Confidence intervals about the mean

Historically, there has been some confusion surrounding the calculation of confidence limits about the mean of a log-normally distributed variable. The main difficulty with confidence limit calculations is that the mean and the variance of the log-normally distributed variable are not independent. As a result, there are several recommended ways to calculate confidence intervals. One study evaluated several methods using Monte Carlo simulation (Parkin *et al.* 1990), and found that a method developed by Land (1973) yields exact confidence limits: the upper and lower limits perform at the stated probability level.

6.4.3 Hypothesis testing

6.4.3.1 Mean versus median

As pointed out earlier, the mean and median of a log-normal distribution have two different values. The choice of the appropriate location parameter is critical in hypothesis testing, as it can affect the conclusions drawn from the data. There are few guidelines regarding the validity of focusing on the mean or median as a summary statistic, or on the sensitivities of statistical tests to differences in the mean versus the median. Often, the median is chosen over the mean because of its resistance to the

extreme values often observed with non-normal distributions. However, because the mean and median convey different information about the population, this rationale is not always valid.

These are the two most frequently used location parameters to summarise log-normal data. It should be recognised that the mean and median of a log-normal distribution actually convey different information about the distribution. Both of these location parameters are indicators of central tendency of the population. The mean is the centre of mass of the distribution, while the median is the centre of probability of the distribution.

In some cases, the median may be a more appropriate indicator of central tendency (Hirano *et al*. 1982; Loper *et al*. 1984; Landwehr 1978); in other situations, the mean is more appropriate (Parkin 1991; Gilbert 1987, p 45-57). The choice of the mean or the median as the summary statistic depends upon the objectives of the experiment, and the nature of the sampling. This choice between the mean and median will dictate the appropriateness of a transformation for normality, and the proper statistical test to use. A major consideration is the influence of sample volume effects on the median.

6.4.3.2 Sample volume effects on the median

The central limit theorem predicts that, regardless of the form of the underlying population, the distribution of sample means approaches normality as the number of samples used in computing the means increases. An illustration of this effect is given by Parkin and Robinson (1992). In natural systems, if the variable of interest is randomly dispersed, collecting large samples has the same effect as bulking or pooling of smaller samples. Thus, for a variable that exhibits a skewed distribution, the distribution becomes more symmetrical as sample volume increases, and the value of the median increases (approaches the value of the distribution mean). This effect was observed for bacterial populations in the rhizosphere (Loper *et al*. 1984). The dependence of the median value on the sample volume is a major factor limiting the use of the median (and associated statistical tests of the median) as a summary parameter.

6.4.3.3 The Median as the Location Parameter of Choice

When is it appropriate to use the median? A classical example illustrating a valid use of the median as a summary parameter exists in the field of economics. Personal income data are skewed, and have been approximated by a log-normal distribution. The median makes a good summary parameter for income data because the samples themselves – the individuals – have identity and significance.

The median income level allows individuals to gauge themselves against other individuals (samples) in the population. In environmental sciences, an excellent example of the appropriate use of the median is illustrated in a study of ice nucleation bacteria on plant leaves (Hirano *et al*. 1982). These investigators analysed 24 to 36 individual leaves, and found that the bacterial distributions on the leaves were log-normally distributed. According to the criterion statement given above, the

appropriate use of the median requires that the samples have identity and significance.

The significance of considering the bacterial populations on the individual plant leaves is given by Hirano *et al*. (1982) in their statement, "The quantitative variability of epiphytic bacterial populations on individual leaves may be an expression of the uniqueness of each leaf as an ecosystem, with one or more environmental or biological characteristics significantly different from that of the neighbouring leaf." They continue: "Since foliar plant diseases occur on individual leaves within a given plant canopy, the mean pathogen population for that canopy is of less importance than the pathogenic population on each leaf."

For trace gas flux, the chambers can vary in size, and typically have no identity or significance. The median is not, therefore, the location parameter of choice, so normalising transformations and statistical tests on normalised data should be avoided.

6.4.3.4 The mean as the location parameter of choice

Often in soil science, what is desired is an estimate of the total magnitude of a given microbial process in the ecosystem. For example, soil denitrification in agricultural systems may be an important mechanism of fertiliser N loss. Soil denitrification measurements exhibit highly skewed frequency distributions. Since the volume of a soil sample collected for denitrification determination typically has no particular significance, and because the median of a soil sample population is functionally dependent upon the volume of the samples, the median will underestimate the mass of N lost via denitrification. A possible exception may be the deliberate targeting of urine patches in grazed pastures. In this situation, if one is interested in characterising the population of urine patches, and not necessarily in estimating denitrification loss from the entire pasture, the median could be used.

The mean (centre of gravity of the distribution) is a better indicator of the total N loss from a particular system. In pollution monitoring, Gilbert (1987) defines the total mass of pollutant at a site as the 'inventory' of the pollutant. If the inventory of the pollutant is the desired summary variable, then the median is the wrong estimator of location, since it will systematically underestimate the total mass of material at the site (for positively skewed distributions). Since the mean should instead be the location parameter of choice, statistical tests of the mean (and not the median) should be used for hypothesis testing.

6.4.3.5 Power of hypothesis testing procedures

Previous sections discussed optimum methods for computing summary statistics of log-normally distributed data. However, many studies typically wish to investigate beyond the estimation of population parameters from sample data. In many cases, sampling is conducted to evaluate treatment effects. The assumption of normality is typically required in the application of standard statistical tests. Non-normality will influence the ability of a statistical test to perform at the stated α level. Non-normality

will also affect the power of a statistical test to detect differences when real differences in the underlying populations actually exist.

The preceding discussion highlighted the fact that, with log-normally distributed variables, there is a choice of location parameters, and that the appropriate choice must be consistent with the objectives and methodologies of the problem under study. After selecting the appropriate location parameter, consideration must be given to the statistical methods used at the hypothesis testing stage. It is imperative that the experimenter who has to analyse positively skewed data understands what is being compared when log-normally transformed data, or nonparametric procedures, are used.

Parkin (1993) evaluated several hypothesis tests for determining differences in means and medians of log-normally distributed variables. He observed that transformation for normality and applying a t-test is a test of differences in medians. Such a procedure is insensitive to any differences between population means. A similar result is obtained when parametric approaches are applied. If the median is the location estimator of interest, this is not a problem. However, if the mean is the location estimator of interest, neither of these recommendations is sufficient. A t-test performed on untransformed data and the confidence limit overlap method were insensitive to differences in population median, but were sensitive to differences in population means.

At any given sample size, the t-test on untransformed data detected differences at a lower frequency than the mean confidence interval overlap method. This latter test was also operating at a Type I[6] error rate substantially less than the nominal α-level at which it was applied. Thus, the mean confidence interval overlap method is a conservative test. For the log-normal case described here – regardless of whether the mean or median is the estimator of interest – at sample sizes of n = 4, very poor power is available. When lower sample numbers are available, the only way to increase power is to apply the tests at higher α levels. An Excel spreadsheet for computing the UMVU estimates of the mean and variance of a log-normally distributed variable along with Land's exact confidence limits of the mean is available from T.B. Parkin.

6.5 Estimation of emission factor (EF)

Emissions factors (EF) – representing the proportion or percentage of the N applied as urine, manure, or fertiliser emitted as N_2O over the course of a growing season or annually – are often calculated from N_2O emissions field data (Cardenas *et al.* 2010; de Klein *et al.* 2006). Values of the EF can be estimated by subtracting the cumulative N_2O emissions occurring in a control treatment where no N was added, and from the cumulative N_2O emissions in a given experimental treatment where N was added, then dividing the difference by the amount of N added. EF values can be calculated using the mean cumulative emissions for each treatment receiving N addition over all replicates, and likewise, using the mean cumulative emissions for the control

[6] True null hypothesis incorrectly rejected.

treatments over all replicates. This will obtain a single EF value for each experimental treatment, but with no indication of variance.

Alternatively, EF values can be calculated for each individual treatment replicate. In this case, cumulative emissions for the control treatment within each block (replicate) should be subtracted from the cumulative emissions for a given experimental treatment within the same block, in order to determine the EF value for that particular treatment and replicate. This procedure allows for calculation of mean and variance of each EF value, and for examining differences in the EF among treatments. In this case, users are referred to the considerations and recommendations discussed in the previous sections on statistical analysis and hypothesis testing.

It is normally expected that N_2O emissions from treatments receiving added N will be greater than emissions from no-N control treatments. However, in cases where cumulative N_2O emissions are greater in the control than in the treatment replicate ($EF < 0$), we do not recommend simply substituting $EF = 0$ for these values. Rather, include the actual value in the subsequent statistical analysis, unless excluding that value as an outlier is justified. It should also be noted that some studies have found non-linear relationships between amounts of N added and N_2O emissions, at least for synthetic N fertiliser addition (e.g. Hoben *et al*. 2011). This implies that EF can vary, depending on N addition rate. Thus, it should be kept in mind that an EF calculated for a single rate of N addition may not necessarily be generalisable to other N addition rates, even within the same management and cropping system.

6.6 Conclusion

Use of chambers to determine soil-to-atmosphere emissions of N_2O is labor intensive and requires collection and processing of relatively large data sets. Due to the inherently variable nature of N_2O emissions and the inherent tendency of chambers to alter the quantity being measured, substantial care is required to optimize analysis of the collected data. Careful consideration of appropriate analysis procedures as discussed in this chapter will ensure that upstream efforts with regard to chamber design, sampling regimes, and other aspects of the methodology will generate the most meaningful and statistically valid results.

6.7 References

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7 HOW TO REPORT YOUR EXPERIMENTAL DATA

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Table 7.1: Summary overview of reporting requirements

	Desirable for Emission Factors (EF)	Desirable for Model development
Experimental site	Latitude, longitude, altitude. Soil type and classification. Previous and current site/soil management. Initial soil chemical conditions (specified depths): available nitrogen (NH_4 and NO_3), total nitrogen and carbon contents, pH. Initial soil physical characteristics: texture, bulk density.	As for EF, plus: Historic information on site/soil management and climatic variables should be reported for at least three years. Site management: weed/pest control; drainage limitations or other relevant aspect. Other relevant aspects of soil fertility. Field capacity or soil moisture release curve, soil conductivity wilting point, soil hydraulic conductivity. Number and depth of soil layers.
Methodology	Details of chamber design and deployment. Treatment details: rates of application; total N and total C inputs; NH_4-N and NO_3-N inputs; dates of application and method of application (pH and DM content of manures, manure type). Trial (statistical) design/replication, number of chambers per plot. Duration of experiment. Number of samples taken to estimate the flux from a single chamber. Chamber closure period. Time elapsed between measurements.	As for EF: For control treatments, all information described for treatment plots should be cleary provided.

	Desirable for Emission Factors (EF)	Desirable for Model development
	Number of background control measurements. Average concentration of background control measurements. N_2O emissions for each sampling date, with indication of variability and associated errors for treatment results.	
Auxillary measurements	Soil temperature for each sampling date at given depth. Air temperature for each sampling date at given height. Total daily rainfall/irrigation. Temperature within the chamber for each sampling date (in soil, if so what depth, and screen air temperature). Soil moisture content for each sampling date. Soil available N (NH_4 and NO_3) at relevant depth as frequently as possible. Bulk density in arable soils at key stages throughout the season (cultivation effects). Total yield/dry matter production for each component of the crop (e.g. straw and grain). Total N export in yield or dry matter production.	As for EF, plus: Daily minimum and maximum temperatures. Daily rainfall intensity information. Daily solar radiation. Daily wind speed. Daily relative humidity. Soil drainage, if available. Seeding system for crops (no tillage, conventional tillage, other). Planting date. Harvest date for crops and cutting/grazing dates for pastures. In the case of pastures, an indication of dominant plant species would be appreciated. If possible, an indication of material left on the field and its composition should be also noted, in relation to the seeding system used.

	Desirable for Emission Factors (EF)	Desirable for Model development
Analysis	Flux calculation method. Equipment details including detector and precision of analyser. For GC determinations provide information on column used, temperatures in detector and oven. Detection limit for the method. Quality control information for gas analysis.	As for EF.

7.1 Introduction

This chapter provides guidance on the minimum requirements for reporting N_2O results of chamber methodologies, to ensure that the soundness/robustness of the results can be verified and that derived emission factors (EF) and/or mitigation technologies can be reliably evaluated.

Reporting with metadata allows researchers around the world to compare the results of studies which have generated emission factors and determined treatment effects. The development and evaluation of process-based models at different scales presents additional challenges for information reporting. Hence, reporting requirements to allow N_2O emission results to be used for evaluating process-based models are also given.

The prime objective of country-specific emission factors is that they may be used to improve the accuracy of to inform national inventories of greenhouse gases. To be accepted, information must be published in refereed journals, as a prerequisite from the Intergovernmental Panel on Climate Change (IPCC). The IPCC is responsible for the acceptance of new EF information into the EF database. So as to obtain reliable information for publication purposes, and to allow comparison of results across the globe, a minimum set of information must be provided, together with the scientific results of specific experiments.

7.2 Information to be reported for generating emission factors

7.2.1 Experimental site

General information on the experimental site should be reported explicitly. This includes:

- Latitude and longitude.
- Altitude.
- Soil type and classification.
- Previous site/soil management, going back at least one year, preferably over three years, and providing information on crop type.
- Initial soil chemical conditions: soil available N (NH_4 and NO_3 content, total nitrogen and carbon contents, pH) at relevant depths.
- Initial soil physical characteristics: texture, bulk density, at relevant depths.

The experimental setup should include a control treatment, so that EF can be calculated and reported explicitly. Treatments, including the control, should be reported in detail, indicating the number of replicates, the exact date of application and treatment applied. Control treatments should be managed under the same conditions as the experimental plots, but should not receive N addition. Reporting on control and treatment plots should include previous management history of crops, grazing and nitrogen.

A key criterium for the IPCC EF database is the length of time for which the information is reported. All experimental information of GHG emissions for EF calculation and incorporation into the EF IPCC database should ideally be based on at least a year of data. However, where it involves estimating emission factors for a specific nitrogen source (e.g., N fertiliser application or a urine patch) the duration of measurements could be shorter, as long as the full N-induced emission envelope has been captured (measurements have continued until the emissions and soil N levels are no longer different from a control treatment). Emissions solely from cropping, or productive seasons, are incomplete, and might not be considered for inclusion in the database. Thus, when emissions from cropping areas are studied, the full year (including fallow periods) or the full crop rotation should be reported. The EF for the entire year should be calculated.

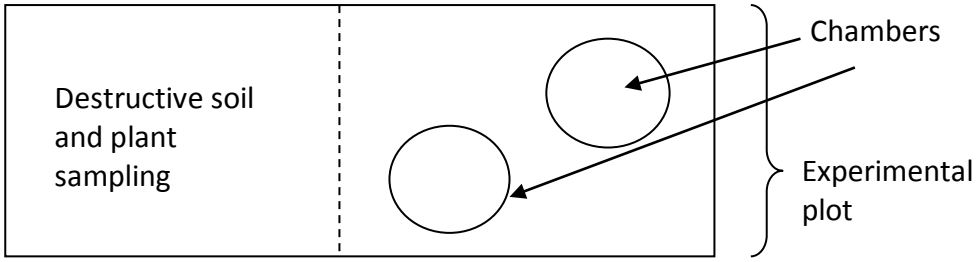
Ideally, more than one year's data should be reported, and measurements should continue until the emissions – or soil mineral N contents – from the treatment plot are not significantly higher than those from the control plot. This is because emissions might be low, due to other factors such as low soil moisture, but if soil mineral N in the treatment plots is still higher than control concentrations, measurements still need to continue to capture any emission that might occur after rain – in other words, capture the entire 'emission envelope'. It is also important that the length of the measurement period is recorded.

7.2.2 Weather and soil conditions

- Average (max and min) soil temperature, to relevant depth for crops or pastures, for each sampling date.
- Average (or max and min) air temperature for each sampling date.
- Total daily rainfall.
- Total daily irrigation, when used.
- Average (max and min) soil and air temperature within the chamber, (when applicable) for each sampling date.
- Soil moisture content for each sampling date.
- Soil available N (NH_4 and NO_3) content at relevant depth for each sampling date if possible, or as frequent as available.

Soil samples, for any associated determination, should be taken as close as possible to, but still outside, the chamber plot, to avoid any soil disturbance for gas determinations. Soil samples taken for nutrients determination should be representative of the area covered. When small experimental plots are used, half the plot can be used for gas determinations, while the other half can be used for destructive sampling of soil and plants (Figure 1) for each treatment.

Figure 7.1: Example of experimental plot layout for greenhouse gases determinations



7.2.3 For N_2O emissions determination

Detailed gas analysis information should be given:

- Details of chamber design and deployment, to ensure they conform with the minimum requirements recommended in these guidelines.
- Number of replicates per treatment, number of chambers per replicate and any additional information regarding blocking.
- Number of samples taken to estimate the flux from a single chamber.
- Time elapsed between measurements for each sampling date.
- Number of background control measurements for each sampling date.
- Average concentration of background control measurements.
- N_2O emissions for each sampling date, with indication of variability (standard error or standard deviation).
- Flux calculation method.
- Equipment details, including detector and precision of analyser. For GC determinations, indicate column used, along with temperatures in detector and oven.
- Detection limit for the method.
- Quality control information for gas analysis.
- Total emissions, with associated error.
- Emission factors, with associated error.

7.2.4 Crop or pasture information

This should include:

- Total yield/dry matter production and components of yield.

- Total N export in yield or dry matter production. If the experiment includes grazing, information on animal type and category, stocking rate and number of grazing days should be included.

This information indicates whether fluxes were measured from a typical level of production, and also allows emission intensities to be calculated.

7.2.5 Treatments

- Application rates (manures: m^3 or t ha^{-1}) - N and C loading rates in fertilisers and manures.
- Fertiliser type (urea or other, liquid or solid).
- Manure type.
- Manure chemical and physical characteristics: dry matter content; pH; total N and C; available N (NH_4^+ and NO_3^-) and uric acid concentration (poultry).
- Application methods (sub surface, injected, slurry surface broadcast, trailing shoe, incorporated, other).
- Application date.
- Detail of control and treatment plot(s) history should be given, and should specify whether subject to same cropping/grazing land use, and previous N inputs.

7.2.6 Statistical analysis

Sufficient information on the uncertainty must be given, using the standard error and number of replicates for experimental design. Also, a clear description of the method of flux calculation and data analysis (see previous chapter on Data Analysis).

7.3 Information required to evaluate process-based models

Because models must be sensitive enough to account for temporal and spatial variability, they require more detailed information on each of the areas mentioned above. Information requirements vary between models, so check with model developers, or documentation, for the necessary model-specific data. Before testing any specific model, appropriate requirements should be discussed among modellers and empirical researchers.

As stated previously, general information on the experimental site location etc. should be reported explicitly. At least one full year of experimental information should be reported (preferably three years if available), so as to account for temporal variability, unless the objective of the model allows for shorter periods of analysis. Ideally, a number of experimental sites will be established over different soil/weather conditions, so as to provide variability for the model to be tested, and make it applicable to different conditions. If possible, this should include a history of site and soil management for the previous three years. Information on weed and pest control, drainage limitations and other site-specific characteristics is also valuable.

Emissions from cropping areas should include fallow periods or full crop rotations.

Weather and soil conditions over the experimental period should include that indicated for EF determinations, plus:

- Daily maximum and minimum temperatures.
- Daily rainfall intensity information.
- Daily solar radiation.
- Daily wind speed.
- Daily humidity.

Crop or pasture information to be reported should include that described for EF, plus:

- Seeding system for crops (no tillage, conventional tillage, other).
- Planting date.
- Harvest date for crops and cutting/grazing dates for pastures.
- Type and number of livestock for grazing events.
- Total yield/dry matter production, including the components of yield (e.g. straw and cereals yields). In the case of pastures, an indication of dominant species would be appreciated.
- Total N export in yield or dry matter production. If possible, an indication of material left on the field, and its composition, should be also noted in relation to the seeding system used.

7.3.1 Statistical analysis

Sufficient information on the uncertainty must be given, using the standard error and number of replicates for experimental design. Also, a clear indication of data analysis used should be noted (see Chapter 6 – Data Analysis). All replicated measurements should be registered and reported on an individual basis per plot (not averaged).

8 HEALTH AND SAFETY CONSIDERATIONS

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The use of chambers to measure N_2O fluxes brings with it a number of health and safety (H&S) risks. It is important that research staff consider these prior to starting any chamber deployment and sampling. Researchers costing the resource requirements of any chamber experiment should budget for H&S considerations: for example, the number of people required to safely sample chambers.

The information in this chapter is not intended as comprehensive. Local site (field and laboratory) conditions should be taken into account, but the following issues should be considered as a minimum when complying with institutional and national legislation, and hazardous substance procedures.

For ease of reference, we have listed the major H&S issues for each stage of an N_2O emission measurement in Table 8.1. Risks therefore follow the same order as the Chapter headings.

Finally, staff should be encouraged to report all accidents and 'near misses' associated with chamber methodology. In this way, systematic accidents can be identified, and procedures put into place before there is any major problem. This reduces future risks to all workers.

Table 8.1: A summary list of potential risks associated with chamber methodology, and guidelines on how to reduce them

Stage	Risk	Consideration
Chamber design	Cuts, lacerations from sharp edges.	Construction material and final design should be selected to minimise sharp edges.
	Fumes from glues used to bond chamber sides.	Any gluing should be conducted in well-aerated rooms, or outdoors.
	Manual handling – muscle strain, back problems, crush injuries.	Bulky and/or heavy chambers should be lifted between at least two people or by machine. Gloves and protective footwear (hard boots) should be worn.
Chamber deployment	Manual handling – muscle strain from installing multiple chambers, crush injuries from using hammers, and lacerations from using sharp implements during chamber installation.	Gloves and hard boots should be worn to avoid injury to hands and feet from hammers and sharp implements when installing chamber bases. Workload should be shared between people to avoid one individual over-straining muscles and joints when installing multiple chambers.
Sample collection, storage and preparation	Muscle strain from repetitive actions, such as bending and use of syringes.	To reduce repetitive injury from repeated actions, workers should avoid rushing by giving themselves sufficient time between sampling multiple chambers . Workloads must be shared. Job rotation should minimise impacts. Chamber design and sampling approach should be considered to minimise the muscular effort required for repeated sampling – the size of needle used can impact on the effort required to fill a syringe, for example. Perhaps set a maximum number of chambers per person per day.

Stage	Risk	Consideration
	Needle-stick injuries.	To minimise injury and illness from needle-sticks, workers should take care when using exposed needles in the field on uneven, sometimes slippery surfaces. When not in use, needles should be in guards at all times. New needles should be used at each sampling occasion to minimise infections from a needle-stick. Workers should leave sufficient time between sampling multiple chambers to avoid rushing. Needles should ideally be thrown away after each sampling and definitely after a needle-stick.
	Personal protective equipment and exposure to sun and cold weather.	Workers should take appropriate precautions to avoid sunburn – by applying sunscreen, wearing a hat and long sleeves – and heat exhaustion. Take plenty of water. Workers should wear sufficient clothing and waterproof footwear to keep warm and dry in cold and/or wet weather.
	Exposure to microbiological agents when dealing with livestock faeces.	Where appropriate, personal protective equipment such as gloves, overalls and face masks should be worn. Any open cuts to the skin should be covered before going into the field.
	Exposure to chemicals.	Researchers should read the material safety data sheets of fertiliser products and inhibitor products before using them in the field. Appropriate personal protective equipment should be used.
	Lone field working.	Working alone cannot always be avoided. Wherever possible, more than one staff member should sample. Where this is impractical, institute-based staff should set up procedures to ensure they know that the field worker is safe, such as scheduled phone calls. The lone worker should take a mobile phone into the field, and ensure that it has signal.
Automated systems	Electrical supplies.	Preferably, all field electrical supplies should be low voltage. Mains voltage supplies must be isolated, or protected by Residual Current Devices, in accordance with legislation.

Stage	Risk	Consideration
	Crushing injuries (moving parts).	Workers should be made aware of moving parts capable of crushing hands, fingers etc. Where appropriate, these moving parts should have guards.
	Manual handling.	Gloves and hard boots should be worn to avoid injury to hands and feet when using hammers and sharp implements when installing chambers. Workload should be shared between people, to avoid one individual over-straining muscles and joints when installing multiple chambers.
	Trip hazards.	Gas lines and electrical cables should be tidied and arranged – in bundles where possible – to minimise potential trip hazards.
	Lone field working.	Working alone cannot always be avoided. Wherever possible, more than one staff member should sample. Where this is impractical, institute-based staff should set up procedures to ensure they know that the field worker is safe, such as scheduled phone calls. The lone worker should take a mobile phone into the field, and ensure that it has signal.
Sample analysis	Manual handling: e.g., gas cylinders.	Where appropriate, use cylinder trolleys and lifts to move gas cylinders. Wear protective footwear.
	Compressed gases, pressure/vacuum; noise.	Use of, and training in, regulators, changing cylinders, cylinder clamps/holders. Good ventilation is essential. Use ear and eye protection where required.
	Chemical exposure.	Use appropriate control measures where chemicals are used, or GC labs are shared within larger chemistry labs. Wear lab coats if exposed to chemicals.
	Ergonomic strain.	Back problems from standing all day: use specialised lab chairs, and perhaps use anti-fatigue matting.

Stage	Risk	Consideration
	Needle-sticks.	To minimise injury and illness from needle-sticks, workers should take care when using exposed needles. The lab environment has more stable walking surfaces than does the field, but can sometimes be slippery. When not in use, needles should be in guards at all times. New needles should be used each day, to minimise infections from a needle-stick. Workers should avoid rushing. All used needles, and any from a needle-stick, should be thrown away in a suitable sharps bin.
	^{63}Ni-ECD operation (radioactive source).	'Wipe test' procedures conducted in accordance with manufacturer's and regulatory authority requirements.
Data Analysis	Muscle strain/repetitive strain injury (RSI).	Ergonomic impact (RSI) from repetitive actions, is a risk, especially in data manipulation. The main precaution is to break work up into manageable chunks, with rest breaks and a chance for different activities throughout the day. Ergonomic mouse and keyboard can be used.
	Monitor glare.	The main controls are anti-glare screens, and taking regular breaks. Keep up to date with optician eye checks.

1

9 GLOSSARY AND ABBREVIATIONS

Term	Description
Flow through/non-flow through chamber	A chamber where gas is emitted into a fixed head-space versus a (typically automatic) chamber where air is circulated to an analyser and (optionally) returned to the headspace.
Steady state/non steady state	N_2O is usually measured in non-steady state chambers with headspace gas concentration building-up over time. Steady state (usually refer to absorption chambers for CO_2 flux where CO_2 is taken up from the headspace).
Non-flow through non-steady state N_2O flux chamber	**Also called: static N_2O flux chamber** Chamber sealed to surface for gas emission determination from gas concentration build up.
Trapezoidal rule	Numerical approximation commonly used as an integration method.
Upscaling	Extrapolation of result (e.g., flux estimate) to a larger scale.
r, SD_r	Repeatability (standard deviation). calculated as a measure of analytical precision from repeated analyses using the same method on replicate air samples (i.e., from the same source) under the same conditions (operator, GC system and laboratory).
R, $SD_{R,}$	Reproducibility (standard deviation) Calculated as a measure of overall precision obtainable with repeated analyses using the same method with and samples under different conditions (different operators, GC systems and laboratories).

10 APPENDICES

10.1 Appendix 1 – Water vapour corrections

If measurements are made with moist air, as a mixing ratio (µmol(N_2O)/mol(wet air), water vapour contributes significantly to the mole of air and so in atmospheric measurements, the mole fraction of tracer (e.g. N_2O) is normally corrected or measured with reference to dry air:

S_g (dry air mole fraction)

χ_g ('measured' wet air mol fraction)

χ_{H2O} mol fraction of water vapour

$$S_g = \chi_g / (1 - \chi_{H_2O}) \qquad \text{A1.1}$$

The correction term is of order a few percent. For example, at 20°C/1 Atm and 80% R.H. water vapour mixing ratio is 19000 ppm and water vapour correction: $d_w = 1/(1 - \chi_{H_2O})$ is ~1.019 and 0.320 ppm N_2O in moist air has a dry air value of 0.326 ppm. If water vapour remains constant during chamber deployment then the flux has the same d_w magnitude of underestimation due to the water vapour dilution effect and the corrected flux is given by a modification of Equation (6) Chapter 4.

$$F_{N_2O} = U d_w (c_e - c_b) \qquad \text{A1.2}$$

Example will 'wet' gas in a vial

For the analytical system shown in Figure 4.2, for a 6 mL exetainer with 2ppm of N_2O in dry air from a standard in a lab at NTP (specified here at 101325Pa pressure and 293K temperature) the vial contains:

PV/RT moles of air: (101325Pa * 6/1e6 m3 vial) / (8.31 J mol^{-1} K^{-1} * 293K) = 0.00025 moles (0.25 mmoles)

At 2 µmol N_2O mol^{-1}(dry air) the vial contains 2 *0.00025 = 0.0005 µmoles (0.5 nmoles) of N_2O

In the analysis we assume that all of the 0.5 nMoles is injected onto the analytical column and is part of the chromatography peak area determined by the ECD.

If wet gas is sampled from a chamber headspace that is also 2 ppm with respect to dry air but it has been collected and stored at 80% R.H. at NTP the vial contains:

PV/RT moles of wet air: (101325Pa * 6/1e6 m3 vial) / (8.31 J mol^{-1} K^{-1} * 293K) = 0.00025 moles (0.25 mmoles)

In that wet air vial (at 80% RH) there is 19 mL L^{-1} volume mixing ratio (~ mmol (H2O) mol^{-1} (dry air)) = 19 * 0.00025 = 0.00475 mmoles H_2O

The amount of dry air is 0.25 - 0.00475 = 0.24525 mmoles dry air or in volume terms (6 – 19*6/1000) mL = 5.89 mL

At 2 µmol N_2O mol^{-1} (dry air) N_2O the vial contains 2 *0.00024525 = 0.0004905 µmoles (0.49 nmoles) of N_2O

In the analysis, we assume that all of the 0.49 nmoles of N_2O are injected onto the analytical column, and all of the 4.75 µMoles water are flushed away to waste. In the chromatography, the ECD sees ~2% less N_2O in the plug of gas carried through the analytical column. If the water vapour is not taken account of, and dry gas is assumed, then for that sample, the mixing ratio would be estimated as 1.96 ppm, instead of 2.00 ppm.

Water vapour flux

It is possible that water vapour concentration will increase in the chamber headspace during deployment due to evaporation from the soil into a warming headspace atmosphere. Under these circumstances of increasing water vapour, the error in the flux will be greater than the instantaneous water vapour dilution.

For example, if conditions change from those above to 22°C/1Atm, by the end of the enclosure period if R.H. is 90%, water vapour mixing ratio becomes 24000 ppm and c_w is 1.024.

$$\mathrm{F}_{\mathrm{N_2O}} = U(d_{we}c_e - d_{wb}c_b) \qquad \text{A1.3}$$

So with d_{wb} = 1.019, d_{we} = 1.024 then for: e.g., c_b=0.33, c_e=0.70 then correction to the flux is ~1.028.

In a field sampling example, taking that 1 mm d^{-1} as a typical evaporation rate (E): into a chamber with ambient air at 20°C/80% RH then that is a volume mixing ratio of water is 18900 ppm

(equivalent to 1876Pa vapour pressure= 13.87 g m^3 Abs humidity = 11.74 ppm (wt) g water kg^{-1} air)

Over 40 min into a 13 cm tall chamber (h) water input = E*t/h
= 0.1 cm d^{-1} * 40/(24*60) d /13 cm *1e6 = 213 µL water L^{-1} air added to background
18871 + 213 = 19084 ppm and humidity increases a small amount to 81%.

Under these circumstances the water vapour flux during closure period should not be a major source of additional error in estimated N_2O flux on top of the initial water vapour dilution error.

10.2 Appendix 2 – Calculating GC performance (example)

This appendix takes the user through a worked example, utilising GC data to calculate GC performance characteristics and a chamber flux detection limit, using equations (1) to (6) in Chapter 4. The N_2O concentrations of three batches of 10 ambient samples, analysed by GC, are given in Table A2.1 below.

Table A2.1: N_2O concentrations (uL L^{-1}) of three batches of 10 ambient samples

Day 1	Day 2	Day 3
0.310	0.310	0.309
0.311	0.314	0.315
0.309	0.311	0.307
0.308	0.311	0.311
0.308	0.309	0.313
0.310	0.310	0.313
0.313	0.309	0.317
0.307	0.311	0.306
0.310	0.310	0.314
0.313	0.309	0.312

A 'Single-Factor ANOVA' is then performed in Excel. A single-factor ANOVA will yield a similar table to that shown in Table A2.2.

Table A2.2: ANOVA table for three groups of N_2O ambient concentration GC data, each containing 10 replicates

Source of Variation	SS	df	MS	F
Between Groups	S_{be}	p-1	$M_{be}=S_b/(p-1)$	M_{be}/M_{wi}
Within Groups	S_{wi}	p(n-1)	$M_{wi}=S_{wi}/p(n-1)$	
Total	$S_{tot}=S_{be}+S_{wi}$			

The ANOVA Table for the data contained in Table A2.1 is given in Table A2.3.

Table A2.3: ANOVA table for three groups of N_2O ambient concentration GC data, eachcontaining 10 replicates

Source of Variation	SS	df	MS	F
Between Groups	1.46969E-05	2	7.34843E-06	1.13947326
Within Groups	0.000174122	27	6.44897E-06	
Total	0.000188819	29		

Following Ellison *et al*. (2009), the repeatability standard deviation – SD_r – is obtained using the equation:

$$SD_r = \sqrt{M_{wi}}$$

Using our example data, SD_r is equal to 0.00254 uL L^{-1}.

A repeatability limit – r – is the confidence interval for the difference between two results obtained under repeatability conditions. It is calculated using the equation:

$$r = t * \sqrt{2} * SD_r$$

where t is the two-tailed Student's t values for the required level of confidence, and the appropriate number of degrees of freedom. Using our example data, r is equal to 0.007 uL L^{-1} , where the t-value is set to 2.052 for 95% confidence interval and 27 degrees of freedom.

Using the repeatability limit – r – calculated from our example, dataset emissions of N_2O into the headspace of a static chamber are only considered as a significant emission when $\partial c > \pm 0.007$.

In order to determine the smallest N_2O flux that can be reliably measured, an example calculation will be will be carried out.

The equation for N_2O flux calculation, measured by the chamber method, is:

$$F_{N2O} = (\partial c / \partial t) * (M/V_m) * (V/A)$$

where ∂c is the change of N_2O concentration in the chamber headspace during an enclosure period ($\mu L\ L^{-1}$); ∂t the enclosure period (h); M the molar mass of N in N_2O ($g\ mol^{-1}$); V_m the molar volume of gas at the sampling temperature and atmospheric pressure ($L\ mol^{-1}$); V the headspace volume (m^3) and A the area covered (m^2). The headspace height is (V/A).

For more detail of chamber flux calculation methods, see the Data Analysis Considerations Chapter. For analysis, we will assume that ∂c has been determined by c_e and c_b, and N_2O concentrations at the end and beginning of an enclosure period, so that we can also write:

$$F_{N2O} = U * (c_e - c_b)$$

As an example: for the term U, we will set ∂t = 0.33 h, chamber height = 0.1 m and diameter = 0.5 m so that [V/A] = 0.1 m, atmospheric pressure = 1 (Atm), air temperature = 10°C at sampling, and units of F_{N2O} and c_b and c_e equal to $\mu g\ N\ m^{-2}\ h^{-1}$ and $\mu L\ L^{-1}$, respectively. Thus, for our calculations, U will be 365 $\mu g\ N\ m^{-2}\ h^{-1}\ \mu L^{-1}\ L$.

If we set the repeatability limit – r – to be equal to $(c_e - c_b)$, then the minimum flux is calculated by the following equation:

$$F_{N2O} = U * r$$

Using our example dataset, the minimum detectable flux is 2.555 $\mu g\ N\ m^{-2}\ h^{-1}$ at the 95% confidence level.

10.3 Appendix 3 – Calculating the minimum detection limits of flux calculation methods (Example)

Step-by-Step Detection Limit Calculations

Table A3.4 outlines the procedure for calculating detection limits for N_2O, CO_2 and CH_4 fluxes when the Quad model is used when four time points are collected at equal time spacing's over the total chamber deployment time of 0.667 h. In this example the chamber used is circular (0.3 m diameter) with a headspace height is 0.15 m, the air temperature is 20^oC, and the atmospheric pressure is 0.965 atm. Application of this procedure for other chamber configurations requires use of appropriate chamber volume and surface area values. Detection limits for the other flux calculation models can be computed using the procedure described here, if the appropriate 'a' and 'b' coefficients (Table A3.5) are applied as described in Step 2.

Step 1. Determine the mean ambient concentration and sampling/analytical variability for each gas component. Collect and analyse 20 to 30 ambient gas samples in the same manner as the chamber headspace samples are collected. Calculate the mean and standard deviation for each gas component. The precision is calculated as the Coefficient of Variation (Mean/Standard Deviation). For this illustration, the experimentally determined mean ambient concentrations and sampling/analytical precisions of N_2O, CH_4 and CO_2 are used.

Step 2. Compute the scaled slope factor (θ) for the Quad model. Theta (θ) is calculated using the regression coefficients shown for the Quad model (4 sampling points) selected from Table A3.5 (a=7.617, b=1.004) along with the chamber deployment time (0.667 h) as illustrated in the equation below:

$$\theta = 7.617 * 0.667^{-1.004} = 10.61 \quad (8)$$

This scaled slope factor (11.44) is the same for all the gases. Note 1: Since the 'a' coefficient (7.617) has units of h^{-2} CV^{-1}, the resulting units of θ are h^{-1} CV^{-1}. Note 2: The scaled slope factors for other models can be calculated in the same manner using the appropriate 'a' and 'b' regression coefficients from Table A3.5. For example to calculate the scaled slope factor for the linear model (with 4 sampling points) values of 'a' and 'b' would be 2.211 and 0.9975, respectively.

Step 3. Compute the slope factor for the individual gases. Multiply the scaled slope factor calculated in step 2 by the mean ambient concentration of each gas. For N_2O this value is 11.44 * 323 = 3695. For CH_4 this value is 11.44 * 1.79 = 20.47. For CO_2 this value is 11.44 * 385.5 = 4409. Note: Units of the slope factors for each gas is the volumetric concentration * CV^{-1}. Thus, the N_2O slope factor has units of nL L^{-1} h-1 CV^{-1}. For CH_4 and CO_2, the slope factors have units of µL L^{-1} h^{-1} CV^{-1}.

Step 4. Compute the positive flux detection limit. The slope factors computed in Step 3 for each gas are multiplied by the sampling/analytical precision (CV) associated with

each gas. For N_2O this value is 3695 * 0.044 = 162.6 nL L^{-1} h^{-1}. For CH_4 this value is 20.47 * 0.071=1.45 µL L^{-1} h^{-1}. For CO_2 this value is 4409 * 0.0014 = 6.17 µL L^{-1} h^{-1}.

Step 5. Compute the negative flux detection limit. Negative flux detection limits for each gas are computed by multiplying the positive flux detection limits by -1. The values are -162.6 nL L^{-1} h^{-1}, -1.45 µL L^{-1} h^{-1}, and -6.17 µL L^{-1} h^{-1}, for N_2O, CH_4, and CO_2, respectively.

Step 6. Convert the flux detection limits to a vol/vol basis to a vol/area basis. The flux detection limits have units of volume gas (nL or µL) per L of chamber headspace air per hour. So, the first step is to multiply the volumetric flux detection limit by the chamber headspace volume (L). For a 0.3 m diameter circular chamber with a chamber headspace height of 0.15 m, the chamber volume is 10.6 L. Multiplying the N2O flux detection limit of 162.6 nL L^{-1} h^{-1} by 10.6 L results in a value of 1723 nL h^{-1}. For CH_4, multiplying 1.45 µL L^{-1} h^{-1} by 10.6 L results in a value of 15.4 µL CH_4 h^{-1}, and for CO_2, multiplying 6.17 µL CO_2 L^{-1} h^{-1} results in a value of 65.4 µL CO_2 h^{-1}. Conversion of these values to soil area units is done by dividing by the surface area covered by the chamber. For a 0.3 m diameter circular chamber, the soil area covered is 0.0707 m^2. For N_2O: 1723 nL N_2O h^{-1}/ 0.0707 m^2 = 24370 nL N_2O m^{-2} h^{-1}. For CH_4: 15.04 µL CH_4 h^{-1}/ 0.0707 m^2 = 218 µL CH_4 m^{-2} h^{-1}. For CO_2: 65.4 µL CO_2 h^{-1}/ 0.0707 m^2 = 926 µL CO_2 m^{-2} h^{-1}.

Step 7. Convert the flux detection limits from a volume/area basis to a mass/area basis. A flux calculated from either linear regression or a non-linear model will have units of nL (or µL) trace gas m^{-2} h^{-1}. As described by Parkin and Venterea (2010), 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:

$$PV = nRT \qquad (9)$$

Where P = pressure, V = volume, n = the number of moles of gas, R = the gas law constant, and T = temperature. 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 K^{-1} , and the corresponding units of P, V, N and T are Atmospheres, Litres, Moles, and Kelvin, respectively. The goal of applying the idea gas law is to convert µL (or nL) trace gas to µMol (or nMol) trace gas. To do this, one must have knowledge of both the air temperature and atmospheric pressure. An example of this calculation for an atmospheric pressure of 0.965 Atm and at 20°C is presented below.

1 µL trace gas * 0.965 Atm / ((0.08206 L Atm Mol^{-1} K^{-1}) * (273 + 20)K) * 1 L/106 µL * 10^6 µMol/Mol = 0.0401 µMol trace gas

Similarly, at an atmospheric pressure of 0.965 Atm and 20°C, 1 nL trace gas = 0.0401 nMol trace gas. Thus, multiplication of the trace gas detection limits calculated in Step 6 by 0.0401 will yield units of nMol (or µMol) trace gas m^{-2} h^{-1}. For N_2O: 24370 nL N_2O m^{-2} h^{-1} * 0.041 = 999 nMol m^{-2} h^{-1}. For CH_4: 218 µL CH_4 m^{-2} h^{-1} * 0.041 = 8.94 µMol CH_4 m^{-2} h^{-1}. For CO_2: 926 µL CO_2 m^{-2} h^{-1} * 0.041 = 37.9 µMol CO_2 m^{-2} h^{-1}. Since each nMol

of N_2O contains 28 ng of N, multiplication of the N_2O detection (999 nMol m^{-2} h^{-1}) by 28 results in a detection limit of 27980 ng N_2O-N m^{-2} h^{-1}. Since each µMol of CH_4 or CO_2 contains 12 µg C, multiplication by 12 yields values of 107 µg CH_4-C m^{-2} h^{-1} and 455 µg CO_2-C m^{-2} h^{-1}. Conversion of the N_2O flux detection limit to units of g N_2O-N ha^{-1} d^{-1} is done by: 1) dividing by 10^9 ng g^{-1}, 2) multiplying by 10^4 m^2 ha^{-1}, and 3) multiplying by 24 h d^{-1}, yielding a N_2O positive flux detection limit of 6.72 g N_2O-N ha^{-1} d^{-1}. If the CH_4 detection limit of 107 µg CH_4-C m^{-2} h^{-1} is divided by 10^6 µg g^{-1}, multiplied by 10^4 m^2 ha^{-1} and multiplied by 24 h d^{-1} a value of 25.7 g CH_4-C ha^{-1} d^{-1} is obtained. Similarly, when the CO_2 detection limit of 455 µg CO_2-C m^{-2} h^{-1} is divided by 10^6 µg g^{-1}, multiplied by 10^4 m^2 ha^{-1} and multiplied by 24 h d^{-1} a minimum detection limit of 109 g CO_2-C ha^{-1} d^{-1} is obtained. The corresponding negative detection limits for each gas species are obtained by multiplication by -1.

Table A3.4: A summary list of potential risks associated with chamber methodology, and. Examples of how the equation for the Quadratic flux calculation method is used to calculate flux detection limits for N2O, CH4, and CO2 when the ambient concentrations and analytical precisions are known (shown in Fig. 3) with 4 time point data and a deployment time of 0.667 hours

Parameter	N_2O	CH_4	CO_2
Mean Ambient Concentration (N_2O, nL L^{-1}; CH_4 and CO_2, µL L^{-1})	323	1.79	385.5
Analytical/Sampling Precision (CV)	0.044	0.071	0.0014
Deployment Time (h)	0.667	0.667	0.667
θ (calculated from Table A3.5, Quad method, 4 time points, 0.667 hour Deployment Time)	11.438	11.438	11.438
Slope factor (θ x Mean Concentration)	3695	20.5	4409
Positive Flux Detection Limit (ppb/h or ppm/h) (Slope factor x CV)	162.6	1.454	6.173
Negative Flux Detection Limit (ppb/h or ppm/h) (-1 x Slope Factor x CV)	-162.3	-1.454	-6.173

Table A3.5: Regression coefficients and coefficient of determination (r2) for regression model relating deployment time (DT) to the scaled slope factors (θ) shown in Tables 5 and 6 of Parkin *et al*. (2012). The exponential model used was: θ = a DT-b. See Parkin and Venterea (2012) for descriptions of the regression models

Flux Calculation Procedure	Regression coefficients		r^2
	a	B	
Three sampling points			
Linear Regression	2.314	1.005	≥ 0.9999
Quad	10.06	0.9904	0.9998
H/M	9.290	1.002	≥ 0.9999
rQuad	7.095	0.9944	0.9998
rH/M	8.369	1.001	≥ 0.9999
Four sampling points			
Linear Regression	2.211	0.9975	≥ 0.9999
Quad	7.617	1.004	0.9998
H/M	6.058	1.035	0.9870
rQuad	8.844	0.9966	≥ 0.9999
rH/M	9.231	0.9820	0.9998
HMR	13.20	0.9973	0.9996