Guidelines for Measuring CH_4 and N_2O Emissions from Rice Paddies by a Manually Operated Closed Chamber Method

Version 1

August, 2015

National Institute for Agro-Environmental Sciences, Japan

Guidelines for Measuring CH_4 and N_2O Emissions from Rice Paddies by a Manually Operated Closed Chamber Method

Version 1, August 2015

Lead authors:

Kazunori Minamikawa (Japan), Takeshi Tokida (Japan), Shigeto Sudo (Japan), Agnes Padre (Philippines), Kazuyuki Yagi (Japan)

Contributing authors:

Prihasto Setyanto (Indonesia), Tran Dang Hoa (Vietnam), Amnat Chidthaisong (Thailand), Evangeline B. Sibayan (Philippines), Yusuke Takata (Japan), Takayoshi Yamaguchi (Japan)

Reviewers:

Kazuyuki Inubushi (Japan), Reiner Wassmann (Philippines/Germany), Tetsuhisa Miwa (Japan), Ngonidzashe Chirinda (Colombia/Zimbabwe)

These guidelines should be cited as:

Minamikawa, K., Tokida, T., Sudo, S., Padre, A., Yagi, K. (2015) Guidelines for measuring CH_4 and N_2O emissions from rice paddies by a manually operated closed chamber method. National Institute for Agro-Environmental Sciences, Tsukuba, Japan.

Acknowledgements

These guidelines were commissioned by the Secretariat of the Agriculture, Forestry and Fisheries Research Council of the Ministry of Agriculture, Forestry and Fisheries of Japan through the international research project "Technology development for circulatory food production systems responsive to climate change (Development of mitigation option for greenhouse gas emissions from agricultural lands in Asia)" (known as the MIRSA-2 project) to support the goals and objectives of the Paddy Rice Research Group of the Global Research Alliance on Agricultural Greenhouse Gases (PRRG-GRA). The authors thank to the provider of unpublished field data, Dr. Seiichi Nishimura (NARO Hokkaido Agricultural Research Center, Japan).

Publisher details

National Institute for Agro-Environmental Sciences
3-1-3 Kannondai, Tsukuba, Ibaraki 305-8604, Japan
Tel +81-29-838-8180; Fax +81-29-838-8199
Copies can be downloaded in a printable pdf format from
http://www.niaes.affrc.go.jp/techdoc/mirsa_guidelines.pdf

ISBN 978-4-931508-16-3 (online)

Disclaimer

While every effort has been made through the MIRSA-2 project to ensure that the information in this publication is accurate, the PRRG-GRA does not accept any responsibility or liability for any error of fact, omission, interpretation, or opinion that may be present, nor for the consequences of any decisions based on this information. The views and opinions expressed herein do not necessarily represent the views of the PRRG-GRA.

Table of contents

Preface

Since Prof. Ralph J. Cicerone and his colleagues have covered rice plants with gas collectors at an experimental rice field located in the University of California at Davis in the late summer of 1980, closed chamber methods have been used for measuring methane emissions from rice paddies at numerous paddy fields in various parts of the world. The database used for estimating emission and scaling factors for methane from rice cultivation in the 2006 IPCC Guidelines compiled more than 1000 data of seasonal measurements by closed chamber methods at over 100 different sites in 8 Asian countries. Closed chamber measurements are being conducted at various paddy fields in these and other countries up to the present date, in order to study mechanisms of material cycling in the ecosystems or to estimate specific emission factors for developing a greenhouse gas inventory.

The research community doing these measurements often discuss about identifying both "best practice" and gaps in the current methodologies of measuring gas emissions, because inter-comparisons of the methods used among different research groups are limited and assessment of the reliability and uncertainty associated with the results have not been comprehensively discussed. The need for standardized guidelines for measuring greenhouse gas emissions from rice paddies have been recognized from these discussions.

The United Nations Framework Convention on Climate Change (UNFCCC) has introduced in the Bali Action Plan in 2007, the actions and commitments of measuring, reporting and verification (MRV), which is now recognized to be one of the most important building blocks to reduce greenhouse gas emissions from different sources. The MRV framework encompasses submitting national greenhouse gas inventories, undergoing international consultation and analysis, and setting up nationally appropriate mitigation actions (NAMAs). For implementing MRV at the local and national levels, standardized guidelines for measuring, and also for reporting and verifying, greenhouse gas emissions are strongly requested to be provided. The methodology registered for Methane emission reduction by adjusted water management practice in rice cultivation at the UNFCCC Clean development mechanisms (CDM) recommends to carry out measurements using the closed chamber method by providing simple Guidelines for measuring methane emissions from rice fields.

This document, "Guidelines for Measuring CH_4 and N_2O Emissions from Rice

Paddies by a Manually Operated Closed Chamber Method", is a product of discussions in the international science communities, especially that in the Paddy Rice Research Group of the Global Research Alliance on Agricultural Greenhouse Gases (PRRG-GRA) since it was established in 2011. Much of the style and composition of the document follows the preceding publication by the Livestock Research Group of GRA, "Nitrous Oxide Chamber Methodology Guidelines".

As mentioned in the Introduction section of the text, the guidelines have been developed to provide "recommended" protocols based on current scientific knowledge. We tried to provide as much scientific evidences that support the recommendations as possible. In addition, we tried to provide a user-friendly structure of the document by conveying practical and technical "know-how," and defining minimum requirements for the measurements. Nevertheless, there still exist some gaps and uncertainties of the methodologies mainly due to current lack of our knowledge. Therefore, we hereby publish this document as version 1, or best practices at this moment, and hope to make revisions in the future by collecting further knowledge and experiences.

July 2015

Kazuyuki Yagi
Principal Research Coordinator
National Institute for Agro-Environmental Sciences (NIAES)

Recommendations

Here we summarize the minimum requirements (written in upright letters) and recommendations (*written in italics*) of each chapter.

Experimental design

Chapter 2 of these guidelines outlines a basic design for comparative field experiments. For best results, it is important to work out a detailed plan and to prepare a field with homogeneous properties before beginning field measurements.

Category	Minimum requirements and *recommendations*
Research objectives	✓ Set research objectives and a plan for their achievement before beginning the field experiment. · *Repeat all measurements multiple times (e.g., over 2–3 years) with the same design to obtain representative estimates of greenhouse gas (GHG) emissions and the average effects of experimental factors in a field.* · *Prepare alternatives or countermeasures in case the experiment does not go as planned.*
Field preparation	✓ Select a field that is homogeneous with respect to agricultural practices (e.g., organic amendment) and soil properties. ✓ Determine a suitable size for individual plots given the research objectives. ✓ To prevent physical disturbance of the soil and artificial CH_4 ebullition when operating the chambers, set up scaffolding in each plot.
Arrangement of replicated experimental plots	✓ Arrange replicated experimental plots according to the predetermined method of statistical analysis (e.g., analysis of variance [ANOVA]). ✓ Avoid pseudoreplication. ✓ Use a post hoc test (e.g., the Tukey-Kramer method) for multiple comparisons. ✓ Use a randomized block design if any heterogeneity exists (e.g., in the chamber deployment sequence). · *Use at most three factors for ANOVA.*

Chamber design

Chapter 3 of these guidelines outlines the features of an ideal chamber that can be used by every researcher. Several design options are acceptable, taking into account local availability of materials and equipment.

Category	Minimum requirements and *recommendations*
Material	✓ Use lightweight material that is break resistant and inert to CH_4 and N_2O (e.g., acrylic and PVC).
Shape and size	✓ Use a rectangular chamber for transplanted rice fields. ✓ The area covered by the chamber (i.e., its footprint) should be a multiple of the area occupied by a single rice hill. ✓ At least two transplanted rice hills should be covered by each chamber. ✓ Either a cylindrical (e.g., made from a trash container) or rectangular chamber can be used in fields seeded by direct broadcasting. ✓ Record the seed/plant density inside the chamber. ✓ Make sure that the chamber height will always be higher than the rice plant. · *Measure at least three points in each plot.* · *Adjust the planting density to one suitable for the chamber size, if the chamber size is already fixed.* · *Use a double- or triple-deck chamber with adjustable height.*
Base	✓ Use a water seal between the base and the chamber to ensure gas-tight closure. ✓ Minimize the aboveground height of the base. ✓ Determine a belowground depth of the base suitable for the soil hardness (e.g., 5-10 cm).
Other components	✓ (1) Install a small fan, (2) install a thermometer inside the chamber, and (3) drill a vent hole and install a vent stopper. ✓ Equip the chamber with a gas sampling port (e.g., a flexible tube connected to a valve) that is separate from the chamber body. · *Install an air buffer (e.g., a 1-L Tedlar® bag) inside the chamber.*

Gas sampling

Chapter 4 of these guidelines outlines a gas sampling schedule and instruments that should be used during chamber deployment to obtain reliable GHG flux data. These procedures and

recommendations should be applied regardless of the chamber shape.

Category	Minimum requirements and *recommendations*
Period	✓ Determine the measuring period according to the research objectives. ✓ The measurement period should encompass the entire rice growing period for the estimation of seasonal emissions of CH_4 and N_2O. · *In accordance with IPCC recommendations, to calculate the N_2O emission factor, measurements should be obtained throughout a year.*
Time of day	✓ Mid-morning during flooded rice-growing periods (measure once daily to obtain the daily mean CH_4 flux). ✓ Measure all treatments at the same timing. · *Daytime during temporary drainage events during the rice growing period.* · *Late morning during dry fallow periods.* · *Measure the N_2O flux concurrently with the CH_4 flux.*
Frequency	✓ At least weekly during flooded rice-growing periods. ✓ More frequently during agricultural management events (e.g., irrigation, drainage, and N fertilization) and some natural events (e.g., heavy rainfall). · *Weekly or biweekly during dry fallow periods.*
Chamber deployment time and number of gas samples	✓ Deploy chamber for 20–30 min during rice-growing periods. · *Obtain at least three gas samples per deployment depending on sampling and analytical performance.* · *Use a longer deployment time (up to 60 min) during fallow periods.*
Instruments	✓ Use a syringe or a pump for gas sampling, depending on the required sample volume. ✓ Use plastic or glass containers for the gas samples, taking into account the allowable storage period. · *Use an evacuated glass vial equipped with a butyl rubber stopper for gas storage.* · *Use a vacuuming machine to prepare evacuated glass vials, instead of manually evacuating the vials.* · *Use a gas replacement method if the use of evacuated glass vials is impractical.*
Notes for	✓ Check the water volume for water seal in the chamber base.

manual operation	✓ Fill soil cracks up with kneaded soil collected from outside the plot. ✓ Prevent water from overflowing the base when the field is drained ✓ Be gentle when placing the chamber on and removing it from the base. ✓ Avoid placing items on top of the chamber and avoid directly touching the chamber body. ✓ Avoid dead volume in the gas sampler. ✓ Store each gas sample in an evacuated vial under pressurized conditions. ✓ Replace the inside air of the chamber after each measurement by tipping it sideways for a few minutes. · *Use an elastic cord to gently bind the rice plants inside the chamber together and then remove the cord before the chamber is closed.* · *Check the degree of inflation of the air buffer bag (if one is used).*

Gas analysis

Chapter 5 of these guidelines outlines a standard method for analyzing GHG concentration using gas chromatography (GC). Typical GC settings and routine operation are described. Stable GC conditions should be maintained for consistent and accurate analysis of the sampled gases.

Category	Minimum requirements and *recommendations*
GC requirements	✓ Use a commercially made GC instrument equipped with a flame ionization detector (FID) and an electron capture detector (ECD) for analysis of CH_4 and N_2O, respectively. ✓ Use packed separation columns to separate the target gas from other gases. ✓ Use pre-cut filters to remove expected contaminants. ✓ Regularly maintain the GC system (e.g., column conditioning).
Gas injection	✓ Use a gas-tight glass syringe or a gas sample loop for manual injection. · *Avoid using a plastic syringe for the direct injection.* · *An automated gas sampler can be used to minimize the volume and stroke errors associated with manual gas injection.*
Standard gas	✓ Calibrate the GC before every analysis. · *Use certified standard gases.*

	• *Use two concentration levels that are outside the expected observed range.*
GC repeatability	✓ Maximize repeatability by fine-tuning GC settings and operation procedures. ✓ Calculate the limit of quantification (LOQ) of the GC analysis by repeated analyses of a gas of known concentration (i.e., 10 × standard deviation).

Data processing

Chapter 6 of these guidelines outlines acceptable methods for calculating hourly GHG fluxes and cumulative GHG emissions from the analyzed gas concentrations.

Category	Minimum requirements and *recommendations*
Calculation of gas fluxes and cumulative emissions	✓ Normally use linear regression of the gas concentration inside the chamber against time to calculate the hourly flux. ✓ Identify the reasons of non-linearity (if exists) for the validation and correction of calculated flux (see Chapter 6.2). ✓ Use trapezoidal integration to calculate cumulative gas emissions from the hourly flux data.
Limit of quantification for gas flux	✓ Calculate the limit of quantification (LOQ) of the gas flux to identify meaningful (i.e., non-zero) flux values (see Chapter 6.3). ✓ Determine how flux data below the LOQ will be handled.

Auxiliary measurements

Chapter 7 of these guidelines outlines auxiliary measurements that provide supporting evidence for interpreting and generalizing (modeling) the observed GHG emissions. In addition, collection of field metadata (i.e., data about field data) is helpful for secondary users of the field data.

Category	Minimum requirements and *recommendations*
Experimental conditions	✓ Collect data on the field location (at minimum, country, province/state, nearest city, and latitude/longitude). ✓ Collect data on weather conditions (at minimum, climate zone, wet/dry seasons, precipitation, and air temperature). ✓ Collect data on the water and soil environment (at minimum, the water supply source, soil taxonomy, total C and N contents, plow

	layer depth, bulk density, and texture). · *Meteorological data collected at a nearby weather station can be used.* · *Collect information on the field drainage condition, especially if water management is a focus of the research.*
Agricultural management practices	✓ Collect records of cultivation history from at least the preceding 3 years [Name of crop(s), number of crops per year, organic amendments (type and rate), and soil water status during fallow periods]. ✓ Record all current agricultural management practices throughout the year (date/duration, method/type, and rate/amount of each management event). · *Measure the surface water depth frequently to ensure proper water management practices (automated sensors and loggers can be used).*
Rice growth and yield	✓ Record the denomination of rice variety. ✓ Measure the yields of grain and straw. ✓ Calculate yield-scaled GHG emissions. ✓ Record disease and insect damage to rice. · *Regularly measure plant height, number of tillers/ears per unit area or per hill, aboveground biomass, and (optional) root biomass.* · *A yield component analysis is helpful for further investigation.* · *Compare rice growth and yield between plants growing inside and outside of chambers.*
Specific measurements	· *Measure soil redox potential and/or soil Fe(II) content during flooded periods.* · *Monitor soil temperature and moisture throughout the year at 1-hour intervals with automated sensors/loggers.* · *Conduct long-term measurements of total carbon and nitrogen contents in the upper soil layer (to at least 30 cm depth).* · *Periodically monitor soil inorganic nitrogen content (ammonium and nitrate).*

Evolving issues

At this time (i.e., the time of production of these guidelines), there is a lack of consensus on some issues, and others have yet to be explicitly considered.

Issue	Current status and prospects
Equipment availability	✓ For various reasons, it is not always possible to procure the required equipment, so measurement procedures need to be flexible and, thus, may not be uniform.
Standard gases	✓ It is sometimes difficult to obtain certified standard gases. ✓ If necessary, standards of the required concentrations can be produced by diluting high-concentration standard gas with an inert gas (He or N_2) with proper checking of the accuracy of the dilution. ✓ Compressed air can be used as a working standard gas after determination of the target gas concentrations.
Chamber transparency	✓ Chamber transparency (or opacity) remains an open question. ✓ Both transparent and opaque materials have advantages and disadvantages, but which type of material is used often depends on what is available.
Chamber area and number of chambers within a plot	✓ The area covered by each chamber (i.e., its footprint) and the number of chambers that should be deployed within a plot depend on the required measurement accuracy. ✓ The larger the chamber area and the greater the number of chambers deployed, the more reliable the gas flux data will be. ✓ However, practically, the chamber area and the number of chambers may be limited by the number of people available to carry out the measurements. ✓ There is no consensus as to what percentage of the plot area should be covered to obtain a representative gas flux value.
Interpolation to fill gaps in the gas flux data	✓ Insufficient gas flux data collected during drainage or after N fertilization may lead to considerable over- or underestimation of total emissions. ✓ Any such gaps in the measurements should be recorded. ✓ The gaps may be filled by interpolation by making some reasonable assumptions.

1. Introduction

1.1. Background and objectives

Rice (*Oryza sativa*) paddies act as an interface for gaseous carbon compounds between the atmosphere and the land. Photo-assimilation of atmospheric CO_2 by rice plants provides staple food for half the world's population (GRISP, 2013), and decomposition of organic materials in the paddy soil can result in the production of CH_4, a potent greenhouse gas and the second largest contributor to historical global warming after CO_2 (Myhre et al., 2013).

Although 90% of the world's rice paddies are located in Asia, they are a globally important CH_4 source (Smith et al., 2014). Estimates based on IPCC guidelines (IPCC, 2006) indicate that CH_4 emissions from rice paddies total 33–40 Tg $year^{-1}$, or 11% of total anthropogenic emissions (Ciais et al., 2013 and references therein). However, these estimates include considerable uncertainty, because of large uncertainties in emission factors and the poor availability of activity data (e.g., water regimes and residue management practices), which can significantly affect emission strength (Blanco et al., 2014).

Field measurements of CH_4 emissions are the basis of CH_4 emissions estimates and a means of evaluating possible countermeasures for reducing emissions. Most field measurements are obtained by the manually operated closed chamber method, because of its ease of implementation in the field due to the low cost and high logistical feasibility of implementation. Drawbacks of the method include low spatial and temporal representativeness of the measured data, which is limited by chamber size and measurement frequency. Alternatively, micrometeorological techniques can provide near-continuous, spatially averaged estimates, but these methods require a large, homogenous field. Consequently, the manual closed chamber method is often virtually the only available option for comparing emissions between experimental plots in which different agronomical practices are used. Therefore, the manual closed chamber method is expected to continue to have a central role, especially in studies investigating management options for reducing CH_4 emissions.

Numerous studies have used the closed chamber method to measure GHGs, and their protocols are reported in the Materials and Methods section of many journal papers. However, the published information is usually limited in nature, and it is difficult for non-experts to carry out these protocols on the basis of the provided descriptions alone. Alternatively, reference can be made to more methodology-oriented documents on chamber design (e.g., IAEA, 1992, Figure 1.1) and the standardized protocol developed for a specific project (i.e. IGAC, 1994, Figure 1.1). To our knowledge, however, no single document comprehensively presents the detailed information necessary for implementing CH_4 emission measurements from rice paddies using the chamber method. Moreover, it should be noted that the recommended protocols for upland fields (e.g., Parkin and Venterea, 2010; de Klein

and Harvey, 2012) cannot be simply applied to rice paddy studies, because the presence of surface water and rice plants significantly alters the physical mechanisms of gas emissions. Thus, the measurement scheme and assumptions used for flux calculations must also differ considerably.

This document, "Guidelines for Measuring CH_4 and N_2O Emissions from Rice Paddies by a Manually Operated Closed Chamber Method" has been developed to provide "recommended" protocol of the closed chamber method for rice paddy studies based on current scientific knowledge. Furthermore, we wish to convey practical and technical "know-how," which is seldom described in detail in journal articles. In addition, we have attempted to define minimum requirements, which may be useful when, for financial or logistic reasons, full implementation of the recommended protocols is not feasible.

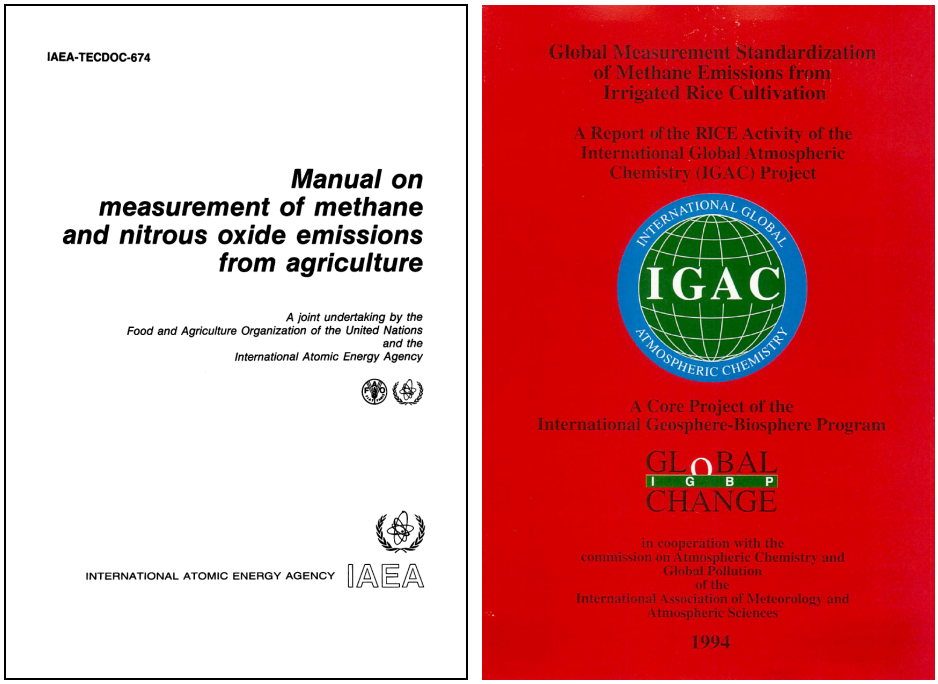

Figure 1.1. Examples of published protocols for the chamber measurement in a rice paddy.

1.2. Biogeochemical mechanisms of CH_4 emissions from rice paddies

In this subsection, we briefly overview CH_4 biogeochemistry in rice paddies because knowledge of them is necessary to establish proper measurement protocols for CH_4 emission by the manual closed chamber method.

1.2.1. Microbial mechanisms of CH_4 production

CH_4 is an end product of the organic C decomposition cascade under anoxic conditions, starting with the hydrolysis of macromolecules (e.g., polysaccharides) and followed by primary and secondary (syntrophic) fermentation to produce hydrogen (H_2), C1 compounds,

or acetate, which then behave as electron donors for CH_4 production (Conrad, 2002). The whole CH_4 production process can be expressed as reduction and oxidation of two molecules of a simple hydrocarbon, one of which is reduced to CH_4 and the other of which is oxidized to CO_2 (Tokida et al., 2010): $2CH_2O \rightarrow CO_2 + CH_4$.

CH_4-producing Archaea (methanogens) are responsible for only the final reaction, i.e., the conversion of simple compounds, mainly H_2 + CO_2 and acetate, to CH_4 (Takai, 1970). Various contingent and collaborative decomposition reactions associated with diverse microbes occur during the course of organic matter (OM) decomposition (Kato and Watanabe, 2010; Schink, 1997).

The proportion of OM converted to CH_4 (rather than CO_2) depends primarily on whether other microbes can harvest more energy by using alternative electron acceptors such as O_2, nitrate, Fe(III), Mn(IV), and sulfate (Takai and Kamura, 1966). If these electron acceptors are available, then microbial competitors of methanogens convert organic C into CO_2, reducing the production of CH_4. As predicted by thermodynamic theory, these microbial competitors can produce energy at lower substrate concentrations, and hence prevail. Fe(III) reducers (geobacters) (Balashova and Zavarzin, 1980; Lovley and Phillips, 1988), in particular, can strongly suppress methanogenesis in paddy soils (Kamura et al., 1963) owing to an abundance of ferric oxides: Fe(III) reduction often accounts for half or more of total electron-donor consumption in paddy soils (Yao et al., 1999). Consequently, organic C oxidation is often coupled with Fe(III) reduction, rather than with methanogenesis, in the early phase of rice growth in irrigated paddies (Eusufzai et al., 2010; Tokida et al., 2010).

The strict requirement of anoxic condition for CH_4 production points to the importance of proper water management; for example, unintended drainage of surface water, even if for a short period of time, may lead to serious and unrecoverable reduction in the rate of CH_4 production and hence the emissions.

1.2.2. Sources of organic matter for CH_4 production

Methanogenesis ultimately depends on primary production and the input of OM into soils. Sources of OM include soil, organic fertilizers, and crop residues (Aulakh et al., 2001; Kimura et al., 2004). The latter two are applied to and subsequently becomes incorporated into the soil. In addition, living rice can be a major source of OM for CH_4 production (Dannenberg and Conrad, 1999; Tokida et al., 2011; Watanabe et al., 1999; Yuan et al., 2012): some portion of the current-season photosynthates is supplied to the soil via either root exudation from living roots or root turnover (sloughing of cells and root decay, collectively referred to as rhizodeposition).

The relative contributions of these sources to CH_4 production depend not only on management practices such as manure application and tillage but also on the rice growth stage (Hayashi et al., 2015). The contribution of applied OM is large during the early rice

growing season, when the rice plants are still small, and the amount of root exudation increases as the rice grows. The root biomass usually peaks at flowering, after which virtually no further roots grow. Therefore, after flowering, root decay may become a major component of rhizodeposition. The relative contribution of soil OM is small compared with the contribution of other sources, but it plays an important role in reducing alternative electron acceptors, most importantly Fe(III). Integrated over the entire growing season, rhizodeposition can account for more than half of total CH_4 production (Tokida et al., 2011; Watanabe et al., 1999).

Because the contribution of rhizodeposition is often very significant, changes in growth and physiology of rice plant from those under ambient condition may lead to divergence in substrate availability and hence may introduce biases in the estimated CH_4 fluxes. Attention is therefore necessary to minimize interfering effects on rice growth during the course of the measurement period.

1.2.3. Emission pathways of CH_4 to the atmosphere

CH_4 produced in paddy soils enters the atmosphere either through aerenchyma tissue of the rice plants (Nouchi et al., 1990; Wang et al., 1997), or ebullition of CH_4-containing gas bubbles (Schütz et al., 1989; Wassmann et al., 1996). Molecular diffusion of dissolved CH_4 across the water-atmosphere can also occur, but the contribution is usually negligible (Butterbach-Bahl et al., 1997; Schütz et al., 1989) because CH_4 is only a sparsely soluble gas (Clever and Young, 1987; Wilhelm et al., 1977) and diffusion in soil solution is four orders of magnitude smaller than in the gas phase (Himmelblau, 1964). In addition, 80–100% of CH_4 diffusing through the oxidative soil-water interface is oxidized by methanotrophic bacteria before reaching the atmosphere (Banker et al., 1995; Frenzel et al., 1992). It is well documented that rice-plant mediated transport is the dominant pathway, accounting for >90% of total emissions when the rice plant develops its root system (Cicerone and Shetter, 1981; Denier van der Gon and van Breemen, 1993; Holzapfel-Pschorn et al., 1986). This fact clearly requires investigators to include rice plants in their chamber measurements; exclusion of rice plants may results in severe underestimation of the estimated CH_4 fluxes.

In rice paddies entrapped gas bubbles (rather than dissolved CH_4 in soil solution) have been shown to represent a major CH_4 inventory, even in soil that is regarded as water-saturated (Tokida et al., 2013). Many studies have shown a very high CH_4 mixing ratio in the bubbles in rice-paddy soils (Byrnes et al., 1995; Holzapfel-Pschorn and Seiler, 1986; Rothfuss and Conrad, 1998; Uzaki et al., 1991; Watanabe et al., 1994). Accordingly release of CH_4-containing gas bubbles can be a major emission pathway at early vegetative stage when the rice plant is still small (Schütz et al., 1989; Wassmann et al., 1996). Also at grain-filling to maturity stages, ebullition could be a dominant pathway because senescence and decay of root system reduce the ability of rice to transfer CH_4 (Tokida et al., 2013).

2. Experimental design

2.1. Introduction

To obtain the best results from a comparative study based on statistical analysis, it is important to work out a detailed experimental design and to prepare homogeneous fields before measurements are carried out. For example, heterogeneous soil properties can mask the effect of experimental factor(s) in the statistical analysis owing to other influential factor(s). Because it can be difficult to prepare homogeneous plots in an actual field, the aim should be to maximize labor efficiency, especially when preparing a new field or conducting a new experiment. This chapter provides basic design recommendations for field experiments and discusses appropriate experimental designs for statistical analysis.

2.2. Research objectives

We conduct field experiments to achieve specific research objectives. Therefore, the objectives should be precisely defined before the experiment is performed. Moreover, to achieve the research objectives, it is essential to prepare an achievement plan before the experiment. For example, to estimate representative GHG emissions and the average effects of experimental factors in a field, we recommended that the measurements be repeated multiple times (e.g., over 2–3 years) using the same experimental design.

Sometimes, an experiment may not go as planned. Therefore, we recommend the preparation of countermeasures and alternative procedures for dealing with problems. Of course, plans can be changed or extended after an experiment has been started, but implementation of the changes may increase soil disturbance or be limited by a lack of materials or space.

2.3. Field preparation

Heterogeneity of soil and field properties (among experimental plots) can confound the effects of experimental factors. For example, different rates of organic amendment in the preceding rice cultivation may alter the amount of carbon substrate available for CH_4 production in the soil (see Chapter 7.3). In addition, the experiment field should be level, especially if water management regimes are being compared among plots. We should therefore select or prepare fields that are homogeneous with respect to agricultural practices and soil properties.

The optimal size of a plot depends on the objectives of the study and on labor availability. For example, it is appropriate to use an entire field as a plot if the aim is to

estimate mean GHG emissions on a catchment or basin scale. On the other hand, the minimum plot area required for comparing the effects of experimental factors is several square meters (e.g., 5 m × 5 m for comparing GHG emissions with rice growth and yield).

Scaffolding should be set up on the plots to prevent physical disturbance of the soil and artificial CH_4 ebullition while measurements are being carried out (Figure 2.1). In addition, to prevent uneven horizontal flow of surface and soil waters, a waterproof sheet can be installed around the edges of each plot (Figure 2.2).

Figure 2.1. Scaffolding (boardwalks) installed for chamber access.

Figure 2.2. Installation of a waterproof sheet around the edges of an experimental plot.

2.4. Arrangement of replicated experimental plots

2.4.1. Introduction

Analysis of variance (ANOVA) is commonly adopted as the statistical technique for comparing

target gas emissions among treatments. Thus, a plot arrangement appropriate for the application of this technique to the data is required. The arrangement should be based on the three principles proposed by Fisher (1926): local control, randomization, and replication. For field experiments to determine GHG emissions, at least three replicates of each treatment should be prepared. Although theoretically two replicates might be adequate for statistical analysis, in practice if only two replicates are used, (1) it is difficult to detect significant differences and (2) editors and reviewers of peer-reviewed journals may doubt the reliability of the measurement data. Statistical significance level is generally set at $p < 0.05$ for GHG studies, but the term "marginal difference" (e.g., $p < 0.1$) may be useful to explain the results with large variation. Here, we give examples of suitable plot arrangements for ANOVA.

2.4.2. Experimental factors

The number and type of experimental factors used for ANOVA are constrained by the plot arrangement. Therefore, when the experiment is being designed, the experimental factors and the appropriate plot arrangement should be considered together.

Table 2.1 defines some statistical terms used in ANOVA. At most three factors should be evaluated by ANOVA in a paddy-field experiment. Although, theoretically, more than three could be evaluated, it is difficult to interpret statistically significant interactions among more than three factors and to arrange plots.

Table 2.1. Explanation of statistical terms in ANOVA

Term	Explanation
Factor	A factor is a selected causal variable that may affect the target response variable (e.g., GHG emissions).
Level	Levels are the different settings of a factor.
Treatment	Treatments are combinations of factors and levels. To evaluate the effects of two factors, each with three levels, nine treatments are necessary. If the effect of only one factor is being evaluated, then the number of levels and treatments is the same.

2.4.3. Randomized block design

Two plot arrangements often used in field experiments are the randomized block design and the split-plot design. A randomized block design (Figure 2.3) is used when some heterogeneity is unavoidable, that is, when it cannot be removed during field preparation. For example, unidirectional surface water flow may be unavoidable in irrigated fields. Such a field should be divided into blocks from the water inlet to its outlet. Another example is the use of multiple fields; in this case, each field is considered a block.

The reason that most often requires an arrangement of randomized blocks to be adopted is sequential chamber measurement, especially when human resources are limited.

Because CH_4 fluxes show substantial diurnal variation (see Chapter 4.3.1), it is often necessary to consider the chamber deployment time as a block (e.g., Figure 2.3). In the illustrated case, a different person is in charge of performing measurements in each row, and the measurements are conducted in sequence from block 1 to block 3 (see Table 4.3 for an example of a detailed time schedule). Note that if there are more than two heterogeneous properties, it may be impossible to interpret the reason of the significant block effect (if one exists) with a randomized block design.

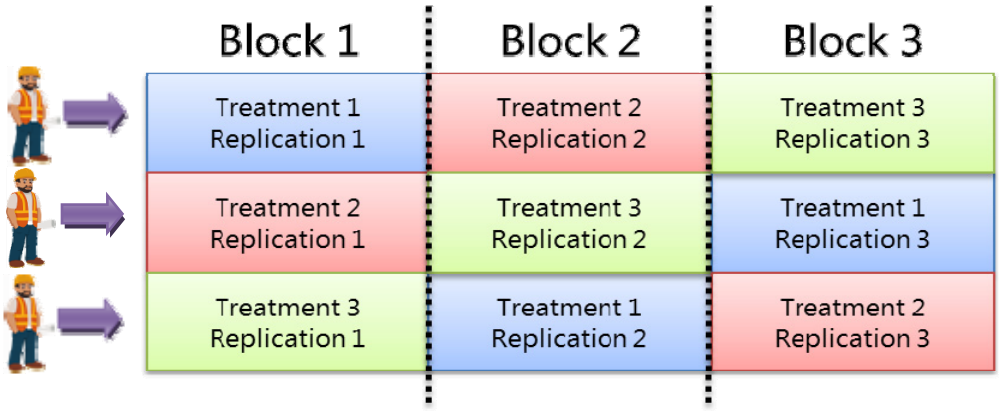

Figure 2.3. Example of a randomized block design for one factor with three levels (treatments) and three replicates.

2.4.4. Split-plot design

A split-plot design is used for a field experiment when the random arrangement of multiple experimental factors is impractical. This design can incorporate blocking, but blocking is not always needed. For example, if the experimental factors being evaluated are water management and fertilizer application rate, a random arrangement is impractical because each treatment would require its own water inlet and outlet. In this case, water management should be considered as a main-plot factor and fertilizer application rate as a sub-plot factor (e.g., Figure 2.4).

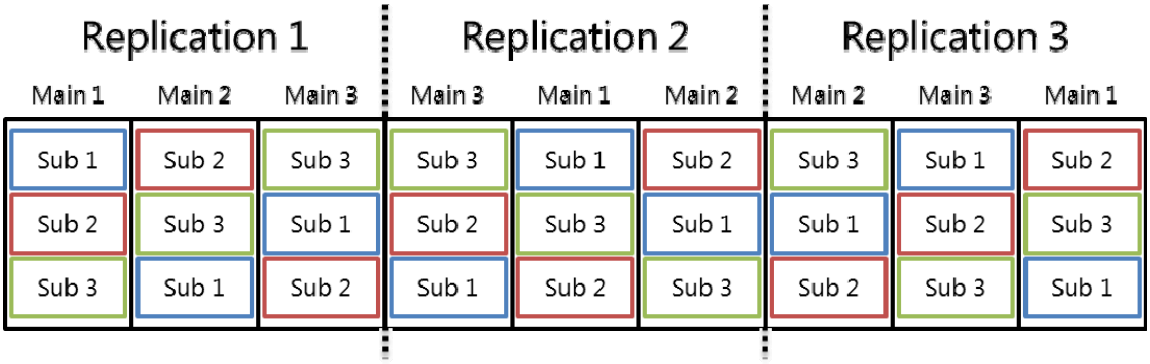

Figure 2.4. Example of a split-plot design for two factors with three main plots, each with three sub-plots, and three replicates.

2.4.5. Completely randomized design

A completely randomized design is the simplest design (Figure 2.5). However, for the reasons described in Chapters 2.4.3 and 2.4.4, this design is seldom suitable for studies of GHG emissions under paddy-field conditions.

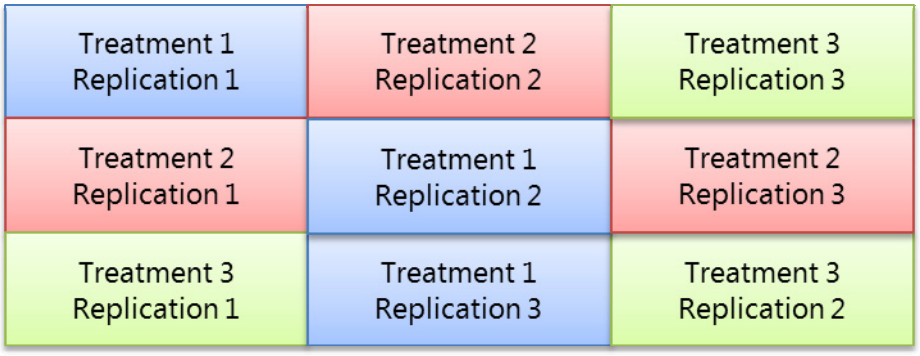

Figure 2.5. Example of a completely randomized design for one factor with three levels (treatments) and three replicates.

2.4.6. Pseudoreplication

We occasionally see published in peer-reviewed journals experiments with an incorrect plot arrangement. For example, in an experiment with one factor and three levels, the combination of the use of one plot for each treatment and the deployment of three chambers within each treatment plot does not provide three independent replicates of each treatment (level) (Figure 2.6). Rather, it is an example of pseudoreplication. Although it is possible to perform ANOVA on the resulting data using PC software, the pseudoreplication makes the ANOVA result meaningless. See Hurlbert (1984) for more examples.

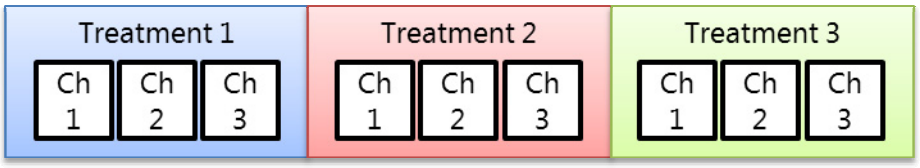

Figure 2.6. Example of an incorrect arrangement of treatment chambers (Ch) in three plots for a one factor experiment with three levels.

2.4.7. Multiple comparisons

Multiple comparisons are comparisons performed after ANOVA to find which means are significantly different from each other. A post hoc pairwise comparison is a typical example. Here we present three parametric methods that are often used for multiple comparisons in peer-reviewed journals (Table 2.2).

Table 2.2. Features of three multiple comparison methods

Method	Features
Tukey-Kramer	✓ Most common and recommended. ✓ Requires homogeneity of variance. ✓ Samples do not need to be the same size. ✓ Result is conservative if sample sizes are unequal. ✓ Small chance of a type I error
Fisher's protected least significant difference (PLSD)	✓ Not recommendable because the possibility of making a type I error is large. ✓ Can be applied when the ANOVA result is significant. ✓ Should not be applied when the number of treatments is 4 or more. ✓ Easy to detect a significant difference.
Duncan's new multiple range test	✓ Not recommendable. ✓ Often used in agricultural research. ✓ Type II errors are unlikely, but the risk of a type I error is high.

2.5. Terminology for experimental errors

Here we follow the terminology of ISO 5725-1:1994 "Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions" as summarized by Wikipedia (Wikipedia contributors, 2015). "Trueness" is the closeness of the mean of a set of measurement results to the actual (true) value, and "precision" is the closeness of agreement among a set of results (Figure 2.7). "Accuracy" is the closeness of a measurement to the true value, and consists of "trueness" and "precision" (Figure 2.7).

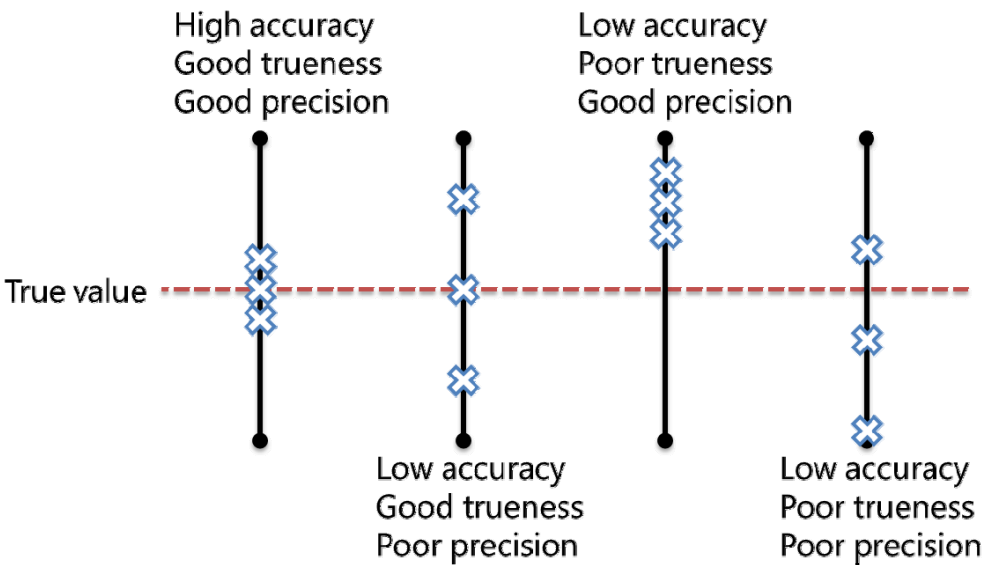

Figure 2.7. Schematic diagram for explaining "accuracy", "trueness", and "precision".

Measurement errors can be divided into two components: random error (*variability*) and systematic error (*bias*). Random error relates to "precision" and is an error in measurement that leads to measurable values being inconsistent when a constant attribute or quantity is measured repeatedly. Systematic error relates to "trueness" and is an error that is not determined by chance but is introduced by an inaccuracy inherent in the system.

"Precision" can be further stratified into "repeatability" and "reproducibility". "'Repeatability' is variation arising when all efforts are made to keep conditions constant by using the same instrument and operator, and repeating during a short time period. 'Reproducibility' is the variation arising using the same measurement process among different instruments and operators, and over longer time periods" (Wikipedia contributors, 2015). See Chapter 5.5 for an example of the repeatability of GC analysis.

3. Chamber design

3.1. Introduction

Ideally, disturbance of the environmental conditions around the rice plants should be avoided during chamber deployment. Provided that such disturbance is minimal, any chamber design is acceptable if it is suitable for the local rice phenology and weather conditions. However, because it is often difficult for various reasons to obtain necessary equipment, we focus here on the minimum chamber design requirements that must be met to obtain scientifically sound measurements. This chapter provides recommendations for preparing an acceptable chamber, focusing in particular on chamber shape. See Chapter 4.7 for notes on manual operations during chamber deployment.

During dry fallow periods, we recommend using low-height chambers to detect small exchanges of CH_4 and N_2O. See Parkin and Venterea (2010) and Clough et al. (2012) for the design of low-height chambers. However, although low-height chambers without covering rice plants may also improve the detectability of small N_2O exchange during a flooded period, its usage is not encouraged because of (1) limited human resource and (2) quantitatively little importance (see Chapter 4.1).

3.2. Material

It is essential to use a material, such as acrylic or PVC, that is inert to the target gases (CH_4 and N_2O). In addition, the material should be lightweight and break resistant. Whether the chamber material should be transparent or opaque is still a subject of discussion (see Chapter 3.6.1). Therefore, we recommend the use of any available material that is otherwise suitable (if possible, acrylic plate) without regard to its degree of transparency.

3.3. Shape and size

The chamber cross-sectional shape often depends on the materials that are available. However, the interior volume of the chamber must be known. Chambers with rectangular cross sections are usually made of acrylic plates (optionally with a stainless steel frame for reinforcement and bonding), whereas one with a round cross section can easily be made from a trash can composed of a suitable material (Figure 3.1). An appropriate thickness for acrylic or PVC plates is usually 3–5 mm.

The larger the area that is covered by the chamber, the more reliable the gas flux data will be. The maximum chamber size is constrained, however, by the need for portability, and its minimum size is constrained by the need to obtain representative measurements and by rice plant height (see Chapter 3.6.2).

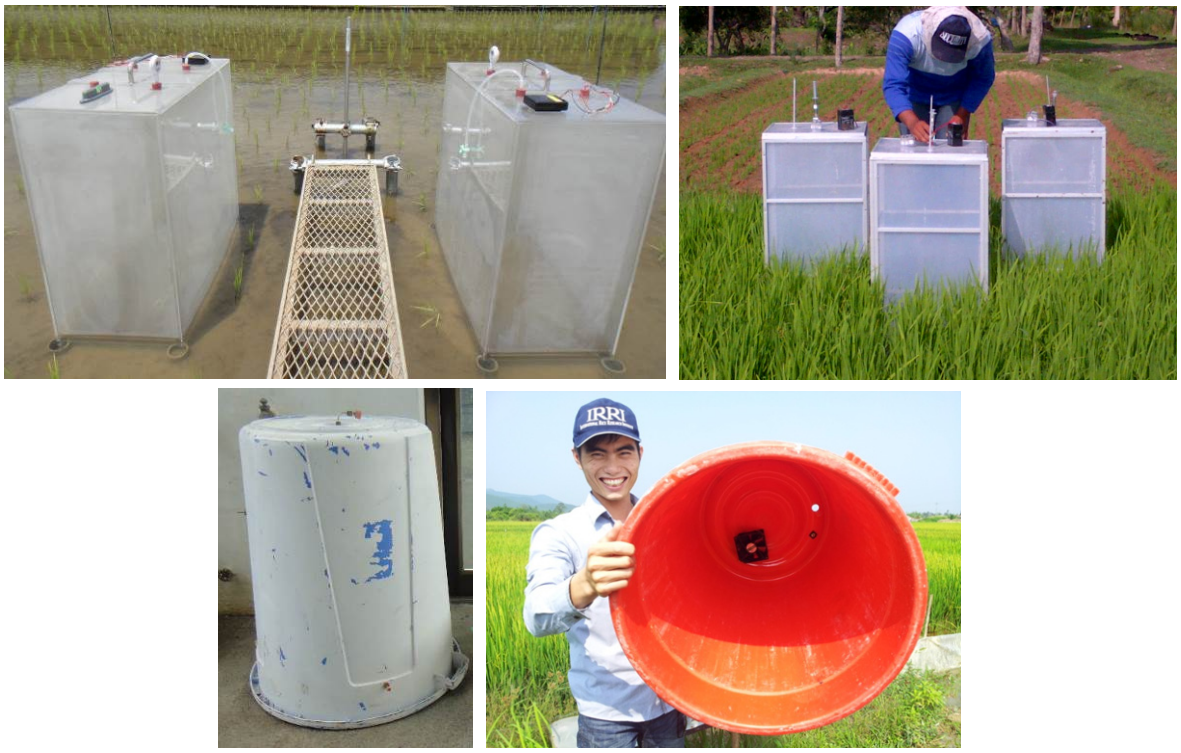

Figure 3.1. Examples of chambers with rectangular or round cross sections.

In general, the method used to sow the rice plants in the field determines the recommended chamber shape. A chamber with a rectangular footprint should be used in transplanted rice fields, and the area it covers should be a multiple of the area occupied by one rice plant (hill). For example, a chamber with a 40 cm × 40 cm footprint is required to cover four hills, each occupying an area of 20 cm × 20 cm (Figure 3.2). This recommendation is consistent with IGAC (1994) recommendations. Otherwise, the area-scaled gas flux will be over- or underestimated, unless a post hoc correction is applied (see Chapter 6.4.1). If the chamber footprint size is fixed, the planting density should be adjusted as necessary to achieve the recommended relationship.

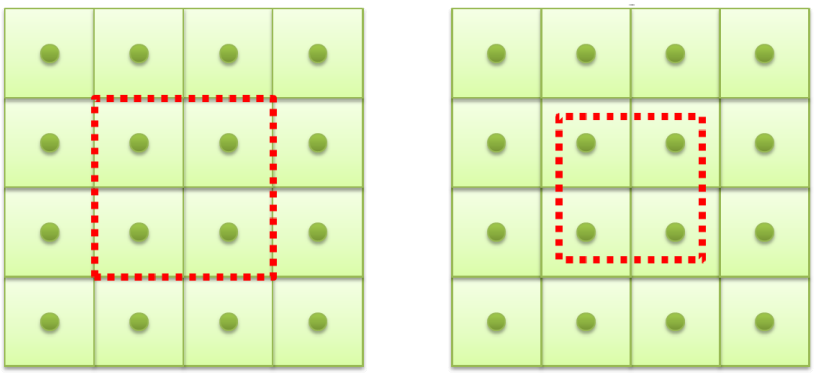

Figure 3.2. Examples of correct (left) and incorrect (right) chamber sizes (cross-sectional area) in a transplanted rice paddy.

With regard to chamber portability, a 60 cm × 60 cm chamber, regardless of its height, is the maximum size that can be carried, even by two people. At least two rice hills should be covered by a rectangular chamber, because the compensatory effect can be expected on rice growth, reducing the spatial variability in the gas flux. Measurement at one point (one chamber) in each replicated plot allows statistical comparison of the plots, but at least three points in a plot are recommended for chambers of the usual size. Having more measurement points (1) enables the spatial variability within the plot to be checked and (2) increases the spatial representativeness of the measurements.

For fields seeded by direct broadcasts, chambers with either a round or a rectangular footprint can be used. However, the actual seed or plant density inside the covered area must be recorded because this information is useful for interpreting spatial variations in the gas fluxes.

The top of chamber should always be higher than the rice plant height so that rice growth will not be suppressed. However, the lower the height of the chamber, the more reliable the gas measurement will be (see Chapter 6.3). Therefore, the use of a double- or triple-deck chamber whose height is adjustable is recommended (Figure 3.3). Although chamber height criteria for upland field plants have been proposed (Clough et al., 2012; Rochette and Eriksen-Hamel, 2008), it may not be appropriate to apply the same criteria to a paddy field. Because a chamber deployed in a paddy is usually equipped with an inside fan, rice height should probably be the primary criterion used to determine chamber height.

Figure 3.3. Examples of double-deck chambers.

3.4. Base

The chamber base (1) provides a gas-tight means of chamber closure and (2) prevents soil disturbance during chamber deployment. The base should be equipped with a water seal to ensure gas-tight closure (Figure 3.4). The base usually remains installed throughout the rice growing period.

The installation of the chamber base inevitably disturbs the environmental conditions around the rice plants to some degree. The aboveground height of the base should be minimal (usually less than 5 cm) so that the base does not interfere with solar radiation. The belowground depth (usually 5–10 cm) depends on the soil hardness and structure, and artificial CH_4 ebullition must be avoided during chamber deployment. Gas leakage through soil crack should be avoided during a (temporal) drained period (see Chapter 4.7). A greater belowground depth may affect rice root growth and soil water and gas dynamics. Four corner pillars (e.g., PVC pipes) inserted as far as the plow pan may help support the chamber when the field is flooded (Figure 3.5).

Figure 3.4. Examples of bases for chambers with round and rectangular footprints.

Figure 3.5. PVC tubes installed in flooded soil.

3.5. Other components

During chamber deployment, the internal environment of the chamber should be maintained under conditions as close as possible to ambient conditions. To achieve this, the inside of the chamber should be equipped with (1) a small fan, (2) a thermometer, (3) a vent hole, and, optionally, (4) an air buffer bag (Figure 3.6).

A small battery-driven fan is used to thoroughly mix the gases in the chamber, so that the target gas concentrations will be uniform (IGAC, 1994). In upland fields, headspace mixing may cause gas flow through the soil (Bain et al., 2005; Xu et al., 2006), but in paddies, a fan should be used because (1) mixing the inside air scarcely affects the air–water–soil gas concentration gradient, (2) rice plants often obstruct air circulation, and (3) little natural mixing occurs in tall chambers.

An air buffer bag (e.g., a 1-L Tedlar® bag) can compensate for both higher air pressures caused by increased temperatures and lower air pressures caused by gas sampling. Although the effect of change in inside air pressure on gas fluxes from a flooded paddy soil remains unsolved, the pressure change should be minimized to maintain the ambient conditions. We therefore recommend using a buffer bag that has been partially inflated before chamber deployment. A vent hole with a rubber stopper is used to prevent drastic changes in inside air pressure during chamber deployment. Chambers used for upland fields occasionally are equipped with thin vent tubes, but their use is still being debated. A vent tube prevents a pressure gradient between the interior and exterior of the chamber from influencing gas exchange (Clough et al., 2012). See Hutchinson and Mosier (1981) for more detailed information on chamber requirements and design.

A thermometer is essential, because temperature data are necessary for calculating hourly gas fluxes (see Chapter 6.2.1). The sensor should not be exposed to direct sunlight. A digital thermometer is recommended because if an analog glass thermometer breaks it can contaminate the soil.

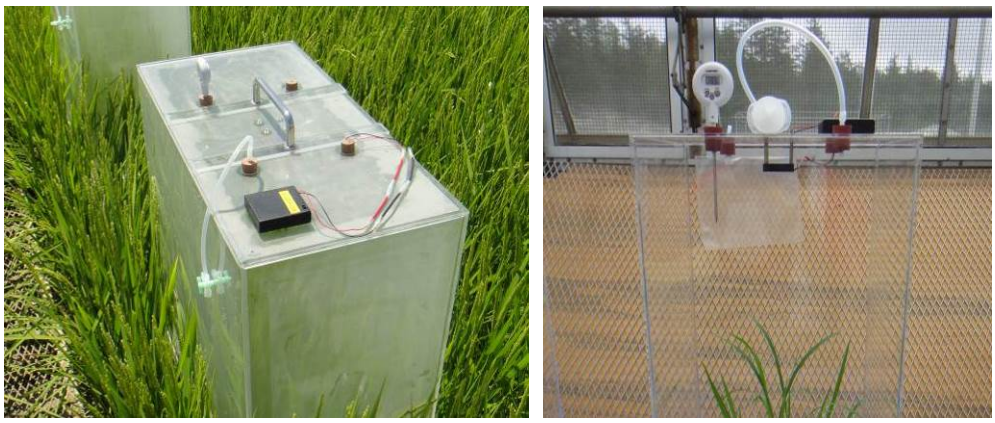

Figure 3.6. Examples of various components installed on the chamber top.

The gas sampling port should be separate from the chamber body to prevent the chamber from possibly being shaken during the sampling. We recommend attaching a flexible tube (20–30 cm long) fitted with a valve to the chamber body (Figure 3.7). The gas within the tube should be replaced by several syringe strokes before each sampling. A ruler (sticker) affixed on the bottom sidewall is useful to easily check the effective chamber height during placement (Figure 3.8).

Figure 3.7. Examples of gas sampling ports connected to the chamber body.

Figure 3.8. Examples of a ruler for reading the effective chamber height.

3.6. Evolving issues

3.6.1. Chamber color

Chamber opacity/transparency remains an open question. Each has both advantages and disadvantages (Table 3.1). In a rice paddy, the chamber covers rice plants through which CH_4 is emitted, so possible effects of chamber opacity/transparency on rice growth and gas fluxes need to be considered. However, better understanding of the relationship between gas fluxes and rice photosynthesis and inside temperature under various climatic conditions is needed to settle this question.

At present, opacity/transparency and shape are often inseparable, and they usually depend on the available material (see Chapter 3.2). In our experience, researchers prefer to use transparent acrylic plates if they are available. The use of opaque acrylic plates in a rice paddy has never been reported to our knowledge. In practice, material availability prevails in selecting between opacity and transparency. However, use of a transparent material is not necessary in the case of drained, unplanted soil; in that case, use of an opaque or reflective material is recommended to prevent temperature increases within the chamber.

Table 3.1. Comparison of opaque and transparent chambers

Subject	Transparent	Opaque
Photosynthesis	Maintained	Restricted
Temperature	Increased	Maintained
Inside visibility	High	None
Chamber operability	High	Low
Material price	High	Low
Material availability	Low	High

3.6.2. Area covered by a chamber vs. plot area

Gas fluxes from a soil generally have high spatial variability, mainly because of heterogeneity of soil properties and rice growth. There is no consensus as to what percentage of the plot area should be covered by chambers to obtain representative gas fluxes from a plot. This problem is relevant to how much accuracy (i.e., the combination of trueness and precision) we require for the estimation of gas emissions.

The percentage of the plot area covered by chambers is determined from the plot area, the chamber area, and the number of chambers deployed in each plot. The use of small plots may not be consistent with research objectives (see Chapter 2.2). Increasing the area covered by each chamber is limited by practical considerations (see Chapter 3.3). The number of chambers deployed simultaneously is limited by human resource availability. As a practical example, if the chamber area is 40 cm × 40 cm, three chambers cover only 1.92% of a 5 m × 5 m plot (and, of course, even less of a larger plot). Sass et al. (2002) measured CH_4 fluxes at multiple points within a rice paddy and estimated that the fluxes were within ±20% of the actual field values within a 95% confidence interval. Khalil and Butenhoff (2008) reported, based on the results of a model simulation, that gas sampling at three points within a field leads to a large uncertainty (40%–60%) in the calculated CH_4 flux. Additional studies are needed to answer the question of what percentage of plot area should be covered by chambers.

4. Gas sampling

4.1. Introduction

There are substantial seasonal and diurnal variations in gas fluxes. We therefore need to consider these dynamics in planning an appropriate schedule of gas sampling. Of course, the more frequent the measurements are, the higher the time resolution will be, regardless of the research objective. This is true in particular when studying how short-term gas flux variations are affected by an agricultural management event. However, the frequency of manual gas sampling is limited by human resource availability and by the need to minimize physical disturbance of the rice plants. Here we provide a practical low-intensity sampling schedule for obtaining gas flux/emissions data with acceptable reliability. In addition, tips about how to best perform manual operations are included. Because the CO_2-equivalent N_2O emissions from a rice paddy are quite low compared to those of CH_4, even under high-N-input conditions (e.g., 11%; Linquist et al., 2012), we prioritize accurate measurement of the CH_4 flux.

4.2. Period

The appropriate period of gas flux measurement depends on the research objectives. Moreover, it may differ between CH_4 and N_2O, even if the objectives are the same, because of differences in emission processes between the two gases. The IPCC (2006) recommended that CH_4 emission factors be applied only during the rice growing period (with notes for wet fallow periods, see Chapter 4.4.3). In contrast, the IPCC recommends continuing N_2O flux measurements for an entire year (including dry and wet fallow periods) to derive the emission factor with comparing N-applied with zero-N plots (IPCC, 2006). Therefore, researchers should determine in advance the measurement period that is adequate for their specific research objectives (Table 4.1).

For example, when measuring both CH_4 and N_2O fluxes to estimate seasonal cumulative emissions, measurements should start before the first agricultural management event of the rice growing season (e.g., tillage, basal fertilization, or organic amendment), before actual rice cultivation begins, and should continue until harvest. We sometimes see seasonal CH_4 flux data in which the initial flux value is already high (i.e., not near zero). Such data are difficult to interpret because the actual start (and end) of the rice growing season cannot be determined. Similarly, a substantial N_2O flux after harvest may be caused by, for example, the incorporation of rice straw into the soil. The definition of the rice growing season depends on local practices, the annual number of rice crops, etc.

Table 4.1. Examples of measuring periods for CH_4 and N_2O in a rice paddy

Objective	Period for CH_4	Period for N_2O
Seasonal cumulative emissions	From an agriculture management event that precedes the rice growing season through the rice growing season until the CH_4 flux ceases after harvest	From an agriculture management event that precedes the rice growing season through the rice growing season until the N_2O flux ceases after harvest
Annual cumulative emissions	Throughout an entire year, including wet/dry fallow period(s) and in the seedling nursery (if one is used)	One entire year
IPCC emission factor	Rice growing season(s)	One entire year
Short-term variation	E.g., several days for diurnal variation, as well as several days during drainage	E.g., several days during drainage, and several days after N fertilization

4.3. Time of day

4.3.1. CH_4 flux during the flooded growing period

CH_4 fluxes vary considerably diurnally — they tend to be high in the daytime and low at night. Figure 4.1 shows a typical diurnal pattern measured after the heading stage in two rice paddies in temperate Japan (Minamikawa et al., 2012). The daily mean flux was obtained at around 10:00 and around 19:00 at both sites. A similar diurnal pattern has also been observed in tropical regions (e.g., in India: Adhya et al., 1994; Satpathy et al., 1997).

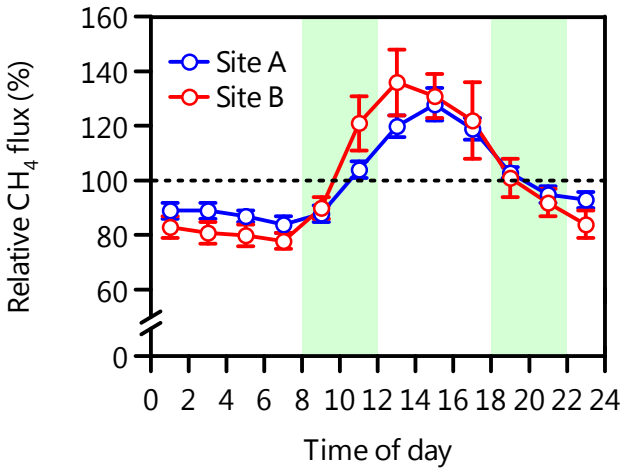

Figure 4.1. Mean diurnal variations of CH_4 fluxes at two Japanese sites (modified from Minamikawa et al., 2012). The dotted line indicates the relative daily mean flux. Bars indicate standard deviations.

How many times a day should gas fluxes be measured? By re-analyzing two datasets of seasonal CH_4 fluxes measured by an automated closed chamber system in Japan,

Minamikawa et al. (2012) determined that measurements performed once per day during mid-morning always resulted in acceptable estimates (i.e., ±10%) (Table 4.2). Therefore, we recommend conducting measurements in mid-morning to obtain the daily mean CH_4 flux. In particular, in temperate parts of Asia, measurement at approximately 10:00 (09:00–11:00) local mean time is recommended. The time window recommended here is consistent with common practice (Sander and Wassmann, 2014). Although twice-per-day measurement can improve trueness (Table 4.2), measurements in the early morning (when the plants may be wet) and at night (when it is dark) in a rice paddy are not recommended. It should be noted that the above analysis was conducted in fields in a temperate climate (in Japan), so further investigation is required to determine the best schedule for fields in other climate regions (see Chapter 4.8.1).

Table 4.2. Effect of the number of measurements per day on the estimation of seasonal CH_4 emissions

Number per day	Site A		Site B	
	Continuous flooding	Midseason drainage	Without rice straw	With rice straw
Once at 08:00-09:59	93	86	87	85
Once at 10:00-11:59	96	93	102	106
Twice (10:00-11:59 and 18:00-19:59)[a]	101	96	112	103
Twice (06:00-07:59 and 12:00-13:59)[b]	102	100	96	101
Three times (06:00-07:59, 12:00-13:59, and 18:00-19:59)[c]	93	91	94	84

Modified from Minamikawa et al. (2012). All times are local time.

Values are total CH_4 emissions estimated as a percentage of emissions measured by the automated closed chamber method.

The measurement interval was weekly in all cases.

Reported by [a] Parkin and Venterea (2010), [b] IGAC (1994), and [c] Buendia et al. (1998).

For various reasons, it may not always be possible to collect gas samples at a fixed time of day. In such cases, as proposed by Sander and Wassmann (2014), the data can be corrected if detailed information on the diurnal pattern in the field (as in Figure 4.1) is available. However, because the actual diurnal pattern on the measurement day cannot be known, we recommend conducting measurements at a fixed time of day if at all possible. The measurement time or times and any correction applied should be reported along with the data.

From a practical standpoint, it is often the case that not all of the chambers can be deployed simultaneously at multiple sampling points, for lack of personnel or because the number of available chambers may be insufficient. In such cases, it is necessary to determine

a suitable chamber measurement sequence within an appropriate time window (Table 4.3). The chamber measurement sequence can be regarded as a block effect in the statistical analysis (see Chapter 2.4.3).

Table 4.3. Example of an appropriate time schedule for one person performing three measurements during a 30-min closure at three positions with three chambers.

Chamber #	Placement	1st sampling	2nd sampling	3rd sampling
1	10:00	10:01	10:16	10:31
2	10:04	10:05	10:20	10:35
3	10:08	10:09	10:24	10:39

4.3.2. CH_4 flux during a temporary drainage period during the growing season

CH_4 stored in flooded soil is released directly to the atmosphere when the field is drained, and its contribution to the total seasonal emissions is often not negligible (e.g., 5-14%, Adviento-Borbe et al., 2015; 6–16%, Weller et al., 2015; 15-16%, Yagi et al., 1996). In addition, Minamikawa et al. (2012) observed no clear diurnal pattern during the non-flooded growing period in two Japanese paddies (Figure 4.2). In such situations, regular measurement at a fixed time of day may not provide reliable results. Therefore, we recommend measuring CH_4 at least once during the daytime; the measurement frequency (i.e., the measurement interval during the growing period) is discussed in Chapter 4.4.1.

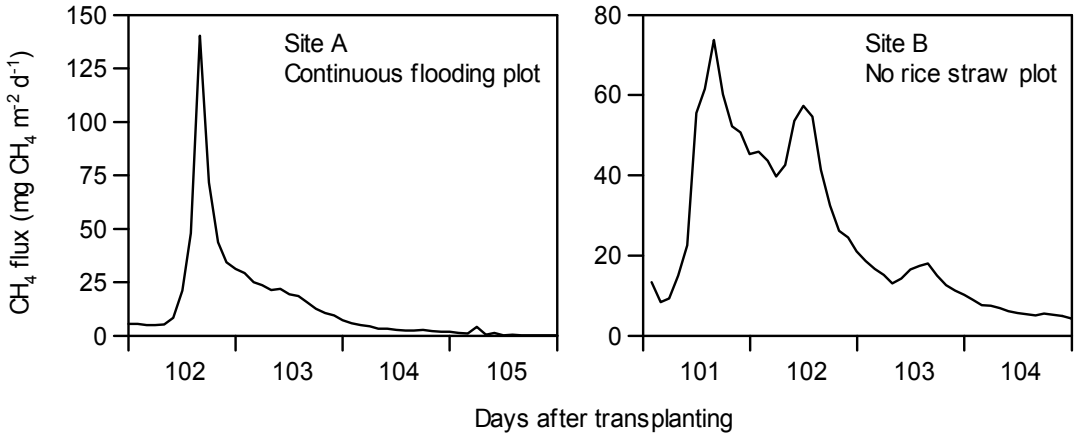

Figure 4.2. Temporal CH_4 flux patterns during a drainage event at two Japanese sites (modified from Minamikawa et al., 2012).

4.3.3. N_2O flux during the flooded growing period

To date, few studies have reported the diurnal pattern of N_2O fluxes from a rice paddy (see Chapter 4.8.1). Hou et al. (2000) reported that N_2O fluxes during the flooded growing period were higher in the daytime and lower in the nighttime, like the diurnal pattern of the CH_4 flux.

However, our preliminary analysis of N_2O flux data measured by the automated closed chamber method in Japan did not show any typical diurnal pattern (Figure 4.3). The lack of a consistent pattern is partly attributable to the application level of N, which is conventionally low in Japan generally (in this field it was 90 kg N ha^{-1}). Therefore, we recommend measuring the N_2O flux at the same time as the CH_4 flux, that is, in mid-morning. For measurement during temporary drainage, see Chapter 4.4.2.

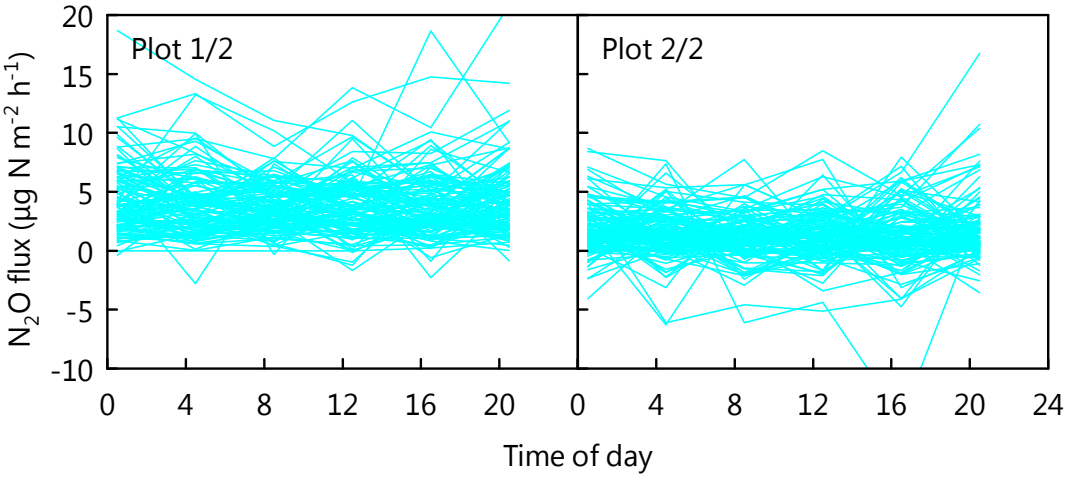

Figure 4.3. Diurnal N_2O flux patterns during the flooded rice-growing period in two plots in Japan (Nishimura, unpublished data).

4.3.4. CH_4 and N_2O fluxes during dry fallow periods

Rochette et al. (2012) suggested that the flux between 10:00 and 12:00 reflects the daily mean N_2O flux in upland fields. Although the diurnal patterns of CH_4 and N_2O fluxes should be determined at each specific site, here, following Rochette et al. (2012), we recommend a late-morning measurement time during dry fallow periods (i.e., the same as the recommended CH_4 flux measurement time during the flooded rice-growing period).

4.4. Frequency

4.4.1. CH_4 fluxes during the growing period

CH_4 fluxes generally increase after flooding because reductive soil conditions develop. Minamikawa et al. (2012) reported that both weekly and biweekly measurement yielded an acceptable estimation (i.e., ±10%) (Table 4.4). However, large fluctuations in the CH_4 flux can occur even under flooded conditions, as a result of, for example, changes in weather conditions. In such cases, biweekly measurement may miss considerable changes in the CH_4 flux. Therefore, we recommend measuring the CH_4 flux at least once a week during the flooded growing period. This measurement interval is consistent with common practice (Sander and Wassmann, 2014).

Table 4.4. Effect of gas sampling frequency on the estimation of seasonal CH_4 emissions

Frequency	Site A		Site B	
	Continuous flooding	Midseason drainage	Without rice straw	With rice straw
Daily	100	99	108	108
Every other day	98	92	103	105
Semiweekly	101	94	104	105
Weekly	96	93	102	106
Biweekly	97	106	104	101

Modified from Minamikawa et al. (2012).

Values are total CH_4 emissions estimated as a percentage of emissions measured by the automated closed chamber method.

Measurements were performed once per day in the10:00–11:59 time window (local time).

As explained in Chapter 4.3.2, drainage events can cause the CH_4 flux to increase sharply because of the direct release of the CH_4 stored in the flooded soil. Therefore, a different measurement-frequency schedule is needed during a temporary drainage period. Minamikawa et al. (2012) reported that measurement at regular intervals did not yield satisfactory flux estimations during the non-flooded growing period, because the measurements did not adequately detect drastic fluctuations in the CH_4 flux over a period of a few days. Therefore, we recommend measuring the CH_4 flux at least every other day during the drainage period until the CH_4 flux ceases (i.e., for 5–7 days). In addition, measurements should be performed just before drainage to obtain a better estimate of the cumulative emissions (see Chapter 6.4.2).

4.4.2. N_2O fluxes during growing period

To our knowledge, no recommendations for a particular frequency of N_2O flux measurement in a rice paddy have been reported. However, the seasonal N_2O flux pattern is known to be event-driven and sporadic. Generally, during flooded rice-growing periods N_2O fluxes remain quite low unless the N input is extremely high. For example, Nishimura et al. (2004) reported that N_2O emissions measured by an automated chamber method during the flooded growing period accounted for 4.3% of the annual emissions (single cropping followed by dry fallow). Therefore, we recommend weekly measurement of the N_2O flux during flooded periods, in conjunction with the CH_4 flux measurement. Although negative N_2O flux values may be obtained during flooded periods, they should be interpreted with due consideration of the gas measurement precision (see Chapter 6.3).

Agricultural management and natural events affecting the N_2O flux include chemical and organic N fertilization, drainage and re-flooding, tillage, and rainfall during fallow periods. These are the same factors that influence soil N dynamics and redox conditions. For example,

flux peaks appear for only a few hours to a few days after flooding following basal N application (Figure 4.4), mainly as a result of bacterial denitrification (Yano et al., 2014). Therefore, we recommend increasing the measurement frequency during these events (e.g., at least every other day until the flux ceases). In addition, for better estimation of cumulative emissions, measurements should be performed just before each agricultural management event (see Chapter 6.4.2).

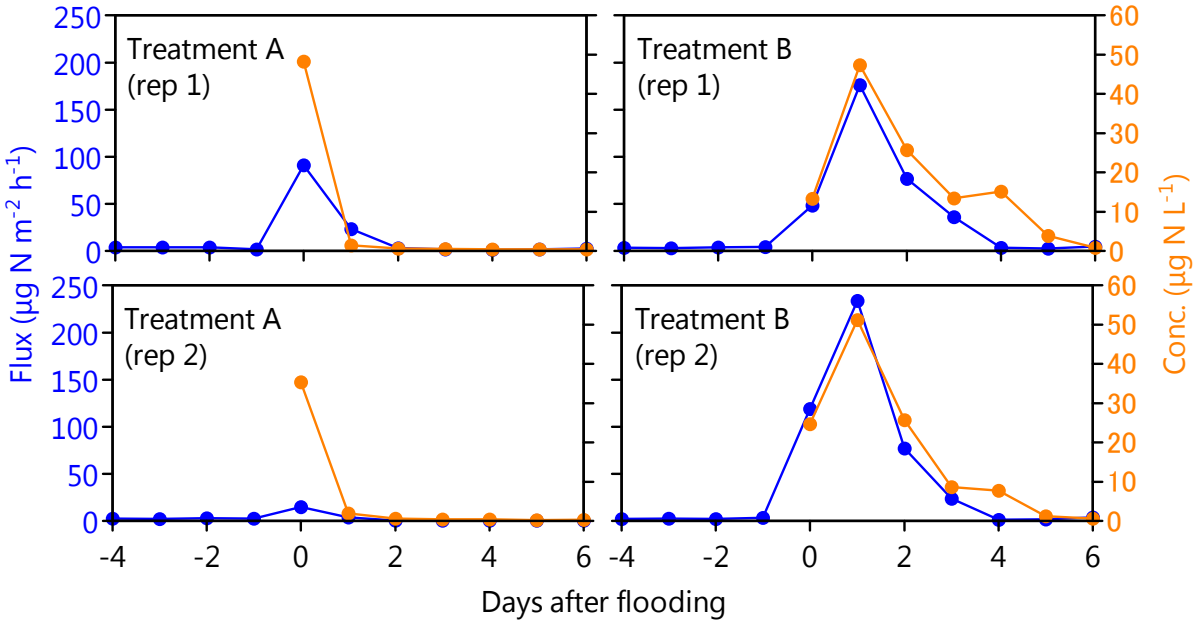

Figure 4.4. Short-term variations in N_2O fluxes and dissolved N_2O concentrations in the surface water after flooding of the field following basal N fertilization at a Japanese site (Nishimura and Minamikawa, unpublished data).

4.4.3. CH_4 and N_2O fluxes during dry and wet fallow periods

Generally, in a dry soil CH_4 is slightly consumed by methanotrophs, whereas a substantial amount of N_2O is produced and emitted. If annual exchanges of CH_4 and N_2O between the atmosphere and soil are being examined, we recommend measuring their fluxes weekly or biweekly during dry fallow periods. It is troublesome if wet conditions occur during fallow periods, such as in a lowland field, during the rainy season, or during a short interval between two consecutive rice growing seasons, because such conditions may cause significant emissions of CH_4 and N_2O. Therefore, we recommend measuring both fluxes frequently during wetting events so that possible peaks will not be missed. IPCC (2006) also recommends obtaining measurements during wet (flooded) fallow periods when estimating the CH_4 emission factor.

4.5. Chamber deployment duration and number of gas samples

The duration of chamber deployment and the number of gas samples collected during each deployment affect the accuracy (and statistical significance) of the calculated gas fluxes (see Chapter 6). A shorter deployment time is preferred for healthy rice growth, because the air temperature becomes elevated within the closed chamber and the CO_2 concentration decreases. On the other hand, a longer deployment time and a greater number of gas samplings improve the accuracy of the flux calculation. According to Sander and Wassmann (2014), most researchers deploy the chamber for 30 min and collect gas samples three or four times per deployment. On the basis of empirical knowledge, we recommend deploying each chamber for 20–30 min during the rice growing period so as to not interfere with rice growth. On the other hand, a longer time (<60 min) is acceptable during fallow periods to improve the accuracy of the gas analysis.

How many gas samples should be collected per deployment? Katayanagi and Tokida (in preparation) analyzed the effect of the number of gas samplings on the precision of the flux calculation by using a Monte Carlo simulation. They found that increasing the number of samplings increases the precision of the flux calculation but the degree of improvement is rather limited (Table 4.5). For example, if errors associated with gas sampling and analysis are 10% (CV), estimated error for the CH_4 flux based on 2 samplings is 22.5% (CV) and that based on 5 samplings is still 19.7%. Here it is noteworthy that the fraction of 'statistically significant' fluxes increases markedly (28.3% for n = 3, and 91.5% for n = 5, Table 4.6), although the actual precision for the estimated fluxes does not change much. These results suggest that the p value of the slope itself does not provide information regarding the precision if the number of sample is different.

Table 4.5. Precision of the estimated CH_4 flux (shown as CV, %) for different number of gas samples per chamber deployment based on Monte Carlo simulation (each for 10,000 runs).

Sampling and analytical error	Number of samples per chamber deployment					
	2	3	4	5	8	10
1%	2.2	2.2	2.1	2.0	1.7	1.5
5%	10.9	11.2	10.5	9.9	8.3	7.7
10%	22.5	22.4	21.1	19.7	17.0	15.6
20%	44.7	45.3	41.7	39.7	33.4	30.7

Modified from Katayanagi and Tokida (in preparation).

In the simulations, we assume that each gas sample has the same degree of relative error associated with gas sampling and analysis regardless of the absolute concentration (i.e. CV is constant).

Results of Katayanagi and Tokida (in preparation) clearly point to the importance of reducing random errors associated with gas sampling and analysis if precision of the estimated flux is to be improved. Decreasing the random error from 10% to 5% is far more effective way than increasing the number of gas samples from 2 to 10 (Table 4.5). If random errors are small, the error of the estimated flux is satisfactory small even when only 2-3 samples are taken. Considering these results, we recommend collecting three samples per 20–30 min chamber deployment. We also suggest that if analytical error is large (e.g. beyond 5%), efforts in reducing the analytical error deserves first priority before taking more gas samples per chamber deployment. However, as a compromise during the improvement of sampling and analytical performance, collecting more than four samples per chamber deployment can be adopted (see also Chapter 6.2).

Table 4.6. Percentage of statistically significant flux (non-zero slope, $p < 0.05$) for the same data-set as in Table 4.5 (10,000 Monte Carlo simulations for each settings).

Sampling and analytical error	Number of samples per chamber deployment					
	2	3	4	5	8	10
1%	–	100	100	100	100	100
5%	–	53.1	98.7	100	100	100
10%	–	28.3	70.9	91.5	99.8	100
20%	–	14.2	30.0	44.6	71.9	82.4

Modified from Katayanagi and Tokida (in preparation).

Note that statistical significance cannot be calculated for 2 points samplings.

4.6. Instruments

4.6.1. Gas collection

Generally, gas samples can be collected from a chamber with a syringe or with a pump (Figure 4.5). The instrument chosen depends partly on the storage container used. A plastic syringe (e.g., 25–50 mL) should be used if the samples are to be stored in (evacuated) glass vials (e.g., 10–30 mL). If the storage container will be a plastic bag, it is necessary to collect a relatively large volume (e.g., >0.5 L) to minimize gas contamination. In this case, a battery-operated pump is helpful, although use of a plastic syringe is also possible. To avoid a drastic change in air pressure inside the chamber, collecting a smaller gas volume is recommended.

Figure 4.5. A plastic syringe (left) and a battery-operated pump (right) for gas collection.

4.6.2. Gas storage

During gas storage, gas leakage and contamination must be avoided. There are three known methods of storing collected gas samples until GC analysis. In each case, it is necessary to check the allowable period of sample storage to avoid significant gas leakage or contamination.

Gas storage in a plastic syringe (the one used for collection) or a plastic bag (such as a Tedlar® bag, Figure 4.6) requires close attention to the possibility of gas leakage or contamination. Generally, the gas permeability of plastic materials is quite high, so a long storage period (e.g., more than 1 day) should be avoided. If long-term storage before GC analysis is unavoidable, we recommend using an evacuated glass vial equipped with a butyl rubber stopper and a cap (a plastic screw top or an aluminum cap; Figure 4.7). Generally, rubber stoppers can be re-used several times; however, their condition should be checked before re-use. Silicone rubber stoppers should not be used, because of their high gas permeability. Note also that butyl rubber stoppers made by different companies differ considerably in gas permeability.

Figure 4.6. Gas being pumped into a plastic bag for storage.

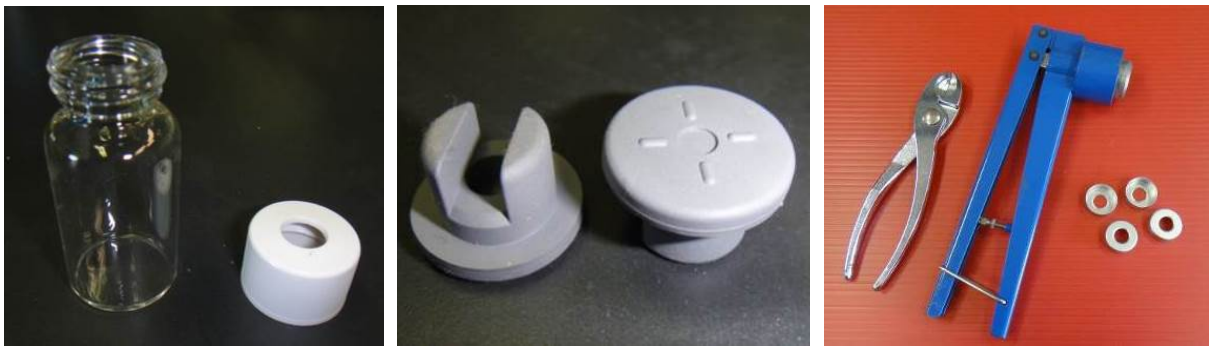

Figure 4.7. A glass vial and plastic screw cap (left); butyl rubber stoppers (middle); and aluminum caps, crimper, and decapper (right).

4.6.3. How to prepare evacuated glass vials

You can prepare evacuated glass vials yourself, as explained below, or you can purchase commercially prepared vials (e.g., Vacutainer®). To evacuate glass vials, an evacuation apparatus and a manometer (Figure 4.8) are needed. The volume of the vial should be much greater than the volume of gas required for analysis (e.g., 10–30 mL if 1–2 mL of gas is needed for the GC analysis).

Figure 4.8. A portable manometer. The needle is inserted into an evacuated vial through a rubber stopper to check the degree of vacuum.

We describe here three types of evacuation apparatus. (1) A vacuum freeze dryer equipped with a stopper-closing function (i.e., trays that can be moved up and down) (Figure 4.9) can be used to prepare ~300 twenty milliliter vials at a time in 30 min. (2) A cylindrical apparatus equipped with an oil pump (Figure 4.10) can be used to prepare 50 twenty milliliter vials at a time in ~10 min (depending on the capacity of vacuum pump). (3) Multiple vials can be connected to a vacuum pump via a vacuum manifold (Figure 4.11).

Figure 4.9. A vacuum freeze drier (left) with two trays for closing the vials with stoppers (right top and bottom).

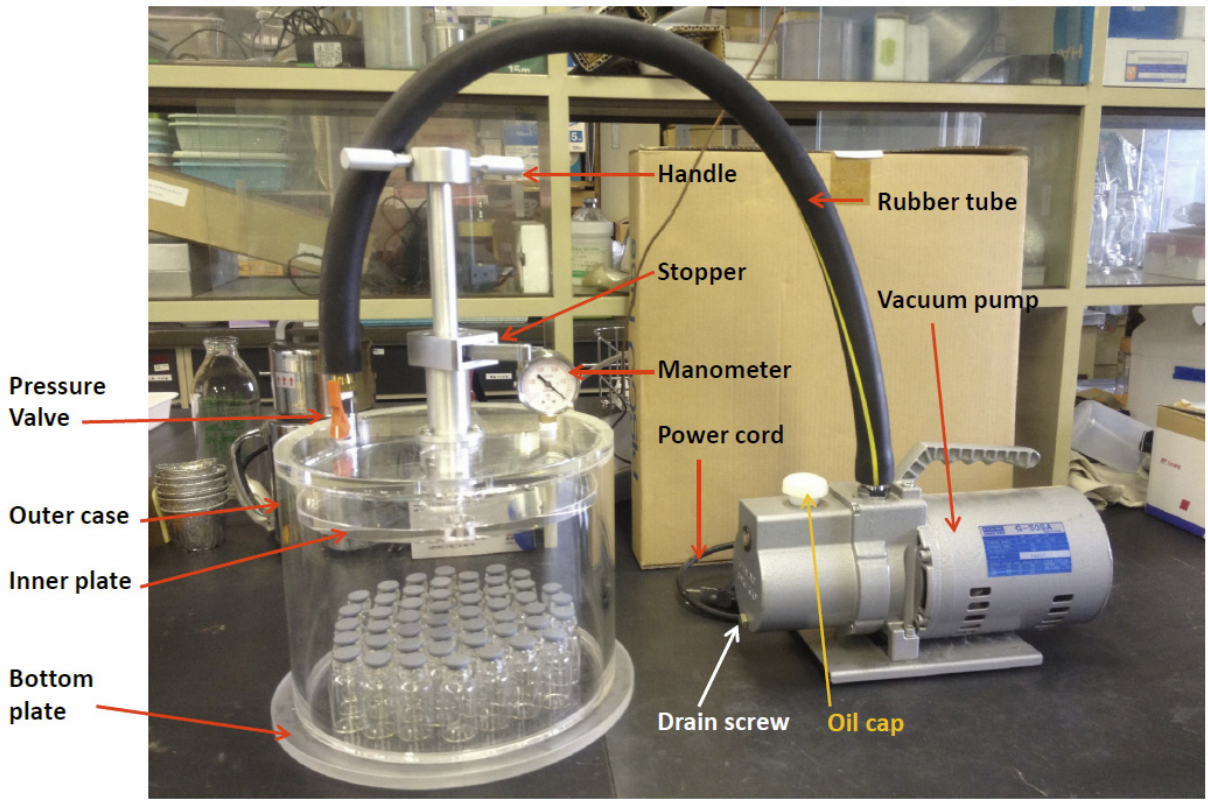

Figure 4.10. An apparatus specifically designed to prepare evacuated vials. After the evacuation is complete, the inner plate is moved down manually to close the vials with stoppers.

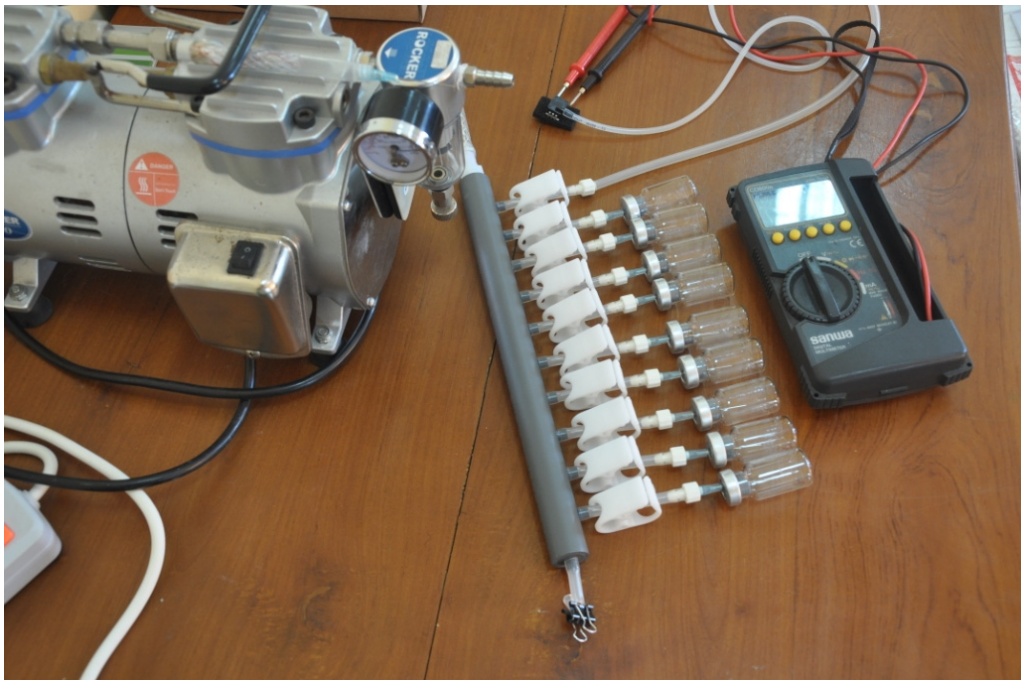

Figure 4.11. Glass vials connected to a self-made vacuum manifold. A manometer monitors the degree of vacuum online.

4.6.4. Gas replacement method

One problem with preparing evacuated vials oneself is that additional equipment is required to create the vacuum. If the necessary equipment is not available, a gas replacement method can be used instead of evacuated vials. In brief, a double-needle technique is used to replace the air in a non-evacuated vial with a sufficient volume of sampled gas (Table 4.7). A tank-in-series model has shown that theoretically gas replacement of more than 4.5 times the volume of the container leaves less than 1% of the original air remaining. However, the actual accuracy will vary depending on the skill level of the operator, so the results should be verified by GC before the actual experiment is carried out.

Table 4.7. A gas replacement procedure with a 10-mL vial used for gas storage

Step	Detail
1	Plug a 10-mL non-evacuated vial with a butyl rubber stopper.
2	First, insert a needle (for degassing) into the vial through the stopper.
3	Second, insert the needle of a 50-mL syringe containing 50 mL of gas sample.
4	Inject 45 mL of the gas sample into the vial, replacing the original air.
5	Quickly remove the first needle.
6	Inject the remaining 5 mL of gas sample to establish a pressurized condition.

4.7. Notes on manual chamber operation

Manual chamber operation consists of the following steps: (1) advance preparation, (2) chamber placement, (3) gas sampling and storage, and (4) chamber removal. Here, we present our recommendations for each step and tips on how best to carry them out.

Advance preparation. Check the water depth in the chamber base to ensure that a water seal will be obtained. If the base is installed and removed every flux measurement, it should be installed at least 24 hours before. If the paddy field is drained, carefully check the interface between the soil and the base and make sure it is airtight. If obvious soil cracks are found, we recommend filling them with kneaded soil collected from outside the plot. In addition, avoid letting water overflow the base when the field is being drained. If the rice plants are tall, it may be hard to cover rice plants with the chamber and extension column without physically disturbing the plants. In such cases, an elastic cord can be used to gently push the rice plants into a bunch; this cord should be removed before the chamber is covered.

Chamber placement. Partially inflate the air buffer bag (if one is used), because both positive and negative pressures may occur inside the chamber (see Chapter 3.5). The chamber should be placed gently on the base to prevent increasing the initial CH_4 concentration by ebullition from the soil. If failed, we recommend removing the chamber and placing it again. In addition, permanent placement throughout the rice growing season should be avoided not to affect adversely rice growth.

Gas sampling and storage. To prevent CH_4 ebullition during sampling, avoid placing measurement components on top of the chamber (see Chapter 3.5). For the same reason, avoid directly touching the chamber body. Note that gas sampling at constant intervals is not necessary for calculation of the gas flux (see Chapter 6.2). Therefore, if the regular sampling time is missed (see Table 4.3), gas can be collected at a different time (which must be recorded). Avoid dead volume in the gas sampler (i.e., syringe or pump) so that the gas concentration in the sampler will be in equilibrium with that in the chamber. If a syringe is used, after it is connected to the chamber via the sampling tube (Figure 3.7), pump the plunger several times to flush the barrel. The collected gas should be stored in an evacuated vial under a pressurized condition. For example, if a 20-mL vial is being used, manually inject ~30 mL of gas while minimizing leakage or contamination; this also allows the gas concentration to be analyzed several times. If a pump is used, the first several seconds of collected gas should be discharged, before the storage container is filled.

Chamber removal. First, the vent plug should be removed and then the chamber should be gently lifted off the base. If failed when the field is drained, water in the base may overflow

and moisten the soil. After the chamber is removed from the base, we recommend tipping it sideways for a few minutes to replace the air inside with ambient air, to prevent an initial high CH_4 concentration during the next deployment of the chamber.

4.8. Evolving issues

4.8.1. Uncertainty of diurnal CH_4 and N_2O flux patterns

There are few reports about the diurnal CH_4 flux pattern in a tropical climate. Therefore, it is not possible to recommend in this chapter a particular time of day for obtaining the daily mean flux in the tropics. Wassmann et al. (2000) reported that significant ebullition occurred at the beginning of rice cultivation in the Philippines, caused both by the application of straw immediately preceding cultivation and by hot weather. This ebullition is not the case for the temperate region. On the basis of a literature survey, Sander and Wassmann (2014) reported that in most studies sampling is carried out in late morning, regardless of the climatic zone. Further investigation is required to elucidate diurnal flux patterns in the tropics and the underlying mechanisms.

Another unresolved issue is the diurnal N_2O flux pattern during the flooded rice-growing period. As mentioned in Chapter 4.3.3, contradictory results have been obtained. The N_2O flux is generally low when the level of N application is low, and thus N_2O flux data from high-N-application fields will be helpful to elucidate the mechanisms underlying the temporal pattern of N_2O flux under flooded conditions.

4.8.2. Effect of human-induced CH_4 ebullition on the number of gas samples

Theoretically, a minimum of two gas samples during each chamber deployment is needed for the flux calculation. Mathematically, two and three gas samples yield the same flux estimation (but not for R^2) if linear regression is used (Katayanagi and Tokida, in preparation). Therefore, it would be possible to recommend two, instead of three, samplings, provided that the skill of the individual doing the sampling and GC performance level had been quantitatively evaluated. This reduction would save labor, enabling the number of replicates (chambers) per time window to be increased. However, although our understanding of the spatiotemporal pattern of natural CH_4 ebullition is improving, in practice, using the concentration data only, it is difficult to explicitly distinguish between human error and a natural event as the cause of ebullition. Accordingly, in the current guidelines we recommend collecting at least three gas samples during chamber deployment. Collection of more samples per chamber deployment and also during the flooded growing period improves our understanding of the timing and field conditions of CH_4 ebullition observed at our own site (see also Chapter 6.2). The accumulation of such fragmentary data will eventually help elucidate the conditions that are necessary and sufficient for adopting the minimum number of samples (i.e., 2).

5. Gas analysis

5.1. Introduction

Several methods are available for analyzing the concentrations of GHGs, including GC with a selective detector, GC with mass spectrometry, GC-less mass spectrometry, and laser-based spectrometry. The optimal method should be selected from the viewpoint of cost, required accuracy (i.e., the combination of trueness and precision), time consumption, and so on. Here we describe the use of GC with a selective detector, because it is the most often used method and it is relatively cheap. This chapter introduces a standard method for analyzing concentrations of CH_4 and N_2O using a GC with a difference detector. Typical settings and routine operations are described.

5.2. GC requirements

5.2.1. CH_4

A flame ionization detector (FID), which uses a hydrogen flame to detect ionized hydrocarbons (HCs), is the most suitable for the detection of CH_4. A FID uses H_2 and air (O_2) as a supplemental fuel and a carrier gas. Atmospheric air contains not only CH_4 but also other HCs, so the FID signal obtained from air is a mixture of signals from CH_4 and other HCs. Therefore, CH_4 and other HCs should be separated from each other so that the target CH_4 concentration can be analyzed precisely.

Packed separation columns are commonly used to separate CH_4 from other components of the gas sample. All of the materials listed in Table 5.1 can theoretically be used to separate CH_4, but with some of these materials, a single analysis may take more than 30 min to complete. On the other hand, materials requiring a shorter time for CH_4 separation may not always ventilate other HCs. Generally, the retention times of CH_4 and other HCs can be shortened by increasing the column temperature. However, higher temperatures (>150°C) risk an increase in signal noise due to the discharge of particulate matter. Therefore, we recommend using a column temperature between 50 and 130°C for CH_4 separation.

The random signal noise level should be reduced to achieve a signal-to-noise (S/N) ratio of more than 10. We recommend the following to reduce the noise level.

- Use He or N_2 (99.999% purity) as the carrier gas.
- Use a charcoal filter to maintain the high purity of the carrier gas.
- Sufficiently dehumidify the air from the compressor used for supplemental combustion in the FID by using a membrane filter and a silica-gel moisture trap.
- Use a catalytic combustor to eliminate HCs contained in this dehumidified air.
- Allow an idling time of at least 30 min after ignition of the FID.
- Even when the FID is not being used, we recommend maintaining a continuous flow of the

carrier gas at a low rate (up to 10 mL min^{-1}).

The installation of a high-throughput analytical system is desirable if a large number of gas samples will be analyzed. The following settings are recommended to complete CH_4 analysis of one sample in 5 min without deploying a "pre-cut" flow-changing technique. (Pre-cut is a method of rapidly venting long-retention-time species with a counterflow of carrier gas by changing the position of gas-flow switching valves.)

- Use a combination of two stainless columns, one packed with Porapak Q (3 mm o.d., 2 mm i.d., 1.5 m long) and the other with Porapak N (3 mm o.d., 2 mm i.d., 1.5 m long). A single 3-m-long column packed with Porapak Q or Porapak N can also be used.
- The optimal particle size of the column fill is 80/100 mesh.
- Set the GC column temperature to 70–90°C. At temperatures below 70°C, the retention times of water vapor and HCs are longer.
- The optimum flow rate of the carrier gas is between 20 and 40 mL min^{-1}.
- Install a moisture trap filled with granular magnesium perchlorate downstream of the gas injection port.
- (optional) Use dual column and detection systems.
- (optional) Use a pre-cut system with a switching valve to prevent the entry of non-CH_4 species into the main separation columns.

Table 5.1. Typical packed column materials for separation of target gases

Materials	System	Target gases
Molecular sieve 5A	Zeolites, porous	inert gases, CO, CH_4
Molecular sieve 13X	Zeolites, porous	inert gases, CO, CH_4
Alumina	Al_2O_3	inert gases, CO, CH_4, CO_2, low-carbon-number HCs
Active carbon	Charcoal	inert gases, CO, CH_4, lower HCs
Unibeads C	Carbon, porous	inert gases, CO, CH_4, CO_2, N_2O
Porapak Q	Polymer, porous	inert gases, CO, CH_4, CO_2, N_2O, H_2O, halocarbons, lower HCs
Porapak N		
Porasil D		

5.2.2. N_2O

The molecular weight of N_2O (44.0) is nearly the same as that of CO_2, so the retention times of both gases are almost the same. Therefore, it is not possible to separate N_2O and CO_2 by using a column packing in which the elution order corresponds to the molecular weight. The two gases have different molecular polarities, however, so separation can be achieved by using a column filler that retains compounds according to their molecular polarity. A thermal conductivity detector (TCD), which is a non-selective detector, is not adequate for measuring

the atmospheric level of N_2O because its concentration in air is only about one thousandth that of CO_2. Therefore, an electron capture detector (ECD), which is a selective detector, is commonly used to measure atmospheric N_2O. Because an ECD has high sensitivity for chemical substances with a relatively high electron affinity, the target N_2O must first be separated from residual species.

A carrier gas of N_2 or Ar is ionized in the ECD cell by a beta ray source (^{63}Ni). In principle, charged electrons of the carrier gas are captured by N_2O with a negative electron affinity and the charged carrier transfer is detected electrically. Therefore, the carrier gas needs to be adequately ionized by the beta ray. It is crucial to first separate contaminants, such as O_2, chlorofluorocarbons, halogens, and oxygen compounds from N_2O in a column, because these contaminants also have a substantial ECD response. It is empirically known that the addition of CH_4 or an Ar-CH_4 gas mixture to the ECD as a make-up gas effectively stabilizes and enhances the N_2O detection response when the carrier gas is N_2. Figure 5.1 shows a typical arrangement of an ECD-GC system for measuring the atmospheric level of N_2O. The major advantage of this system is perfect separation of atmospheric level of O_2, because its long retention time (so-called tailing peak) can overlap the peak of N_2O.

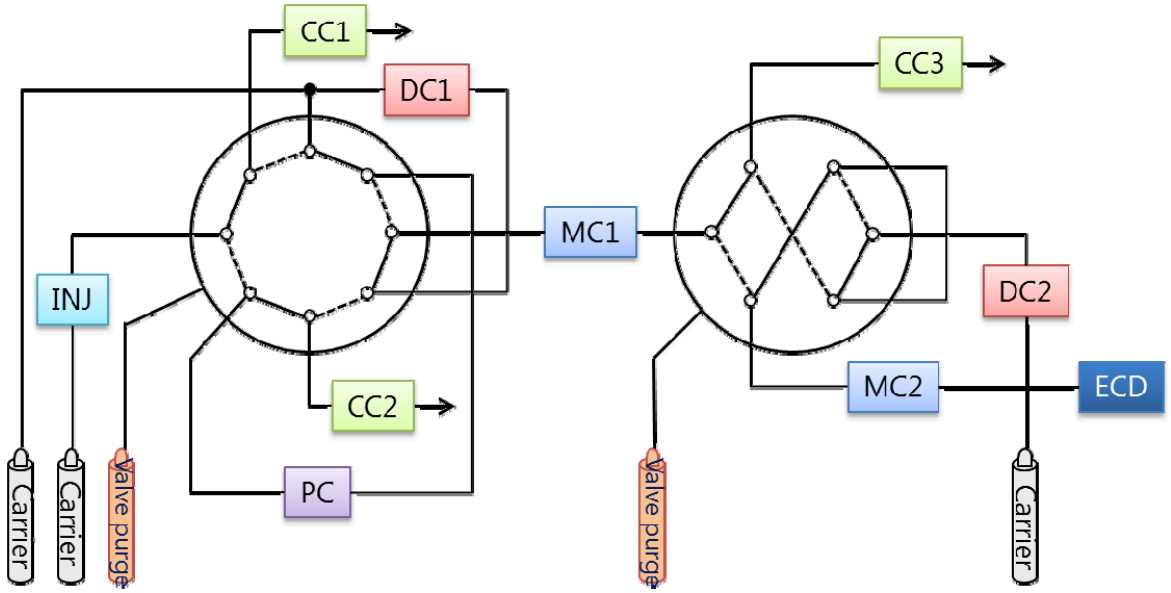

Figure 5.1. A typical ECD-GC arrangement for accurately separating N_2O. Residual contaminants with a long retention time are discharged from the ventilation flow (CC2), which is controlled by counterflow in a pre-cut column (PC). Oxygen and CH_4 that enter the main column 1 (MC1) before the first valve's switching time of 1.5 min after injection are introduced into another discharge flow path (CC3), followed by the second valve switching 2.5 min later to introduce N_2O into the main column 2 (MC2). By a three-stage separation technique, N_2O is separated into MC2. The same column filler must be used in PC and DC1 and in MC2 and CC3. The function of columns CC1, CC2, and CC3, is to compensate for the column-end pressure caused by changing of the switching valve position.

The following ECD-GC settings are recommended for measuring the atmospheric level of N_2O.

- Use N_2 (purity > 99.999%) or a mixture of 95% Ar (purity > 99.999%) and 5% CH_4 as the carrier gas.
- Purify the carrier gas with a charcoal filter and a moisture filter.
- Set the temperature of the ECD to >300°C to obtain a sufficient N_2O peak.
- We recommend using Porapak Q (4 mm o.d., 3 mm i.d., 1.0 m long) or Porapak N (4 mm o.d., 3 mm i.d., 1.0 m long) columns, with a total column length of 3–5 m (i.e., the total length of the eight columns in Figure 5.1), to analyze N_2O in one sample in 10 min.
- The optimal particle size of the column fill is 60/80 or 80/100 mesh.
- Set the flow rate of the carrier gas to between 20 and 40 mL min^{-1}.
- Set the column temperature to between 70 and 90°C.

When the column temperature is less 70°C, some water vapor and HCs will persist in the column, increasing the analytical time to longer than 10 min. On the other hand, it is difficult to separate the N_2O completely if the column temperature is higher than 90°C.

5.2.3. Maintenance

Continuous good operation of GCs requires maintenance, such as replacement of the moisture absorbent (silica gel) and the rubber septum of the injection port, cleaning the gas-flow channel, and column conditioning (Figure 5.2). Routine procedures should be carried out at regular intervals.

Figure 5.2. Examples of GC maintenance. Left, glass tube cleaning; top right, replacement of silica gel; bottom right, checking the water level in the hydrogen generator.

Column conditioning is required in a non-flow-change system (i.e., no pre-cut or back-flush system) to remove the residual contaminants that gradually accumulate in the column. If conditioning is not performed regularly, the baseline of the GC will be unstable. Therefore, we recommend performing the conditioning regularly after a certain number of gas sample injections. The conditioning temperature of the column should be lower than the column's maximum limit, while taking into account the temperature limits of other instruments. The conditioning should be conducted with a continuous carrier gas flow for about 24 h to ventilate residual species.

5.3. Gas injection

It is necessary to minimize fluctuation of the gas injection volume to reduce the uncertainty of the gas analysis (see Chapter 5.5). Several gas injection methods are commonly used, such as manual injection using a glass syringe, manual injection using a gas sample loop, and an automated injection system (ready-made or custom-made). If a gas injection port is used, the rubber septum of the GC port should be replaced after every 100 samples to avoid contamination by the ambient air.

For manual gas injection, the use of a gas-tight glass syringe (ideally equipped with an open-shut valve/screw) is recommended (Figure 5.3). Use of a glass syringe with an open-shut valve allows the gas sample to be stored in a pressurized state — the pressure is released just before the injection by opening the valve. A plastic syringe is not recommended for manual gas injection because precise reading of the scale is difficult and gas leakage may occur. A sample loop with a known volume can also be used for manual gas injection (Figure 5.4). A sample loop is particularly useful when a glass syringe is not available. An automated gas sampler can minimize errors in stroke volume (Figures 5.5 and 5.6). However, an automated system is expensive, and the injector (syringe and needle) sometimes must be replaced because of damage caused by a system malfunction.

Figure 5.3. A gas-tight glass syringe with an open-shut valve.

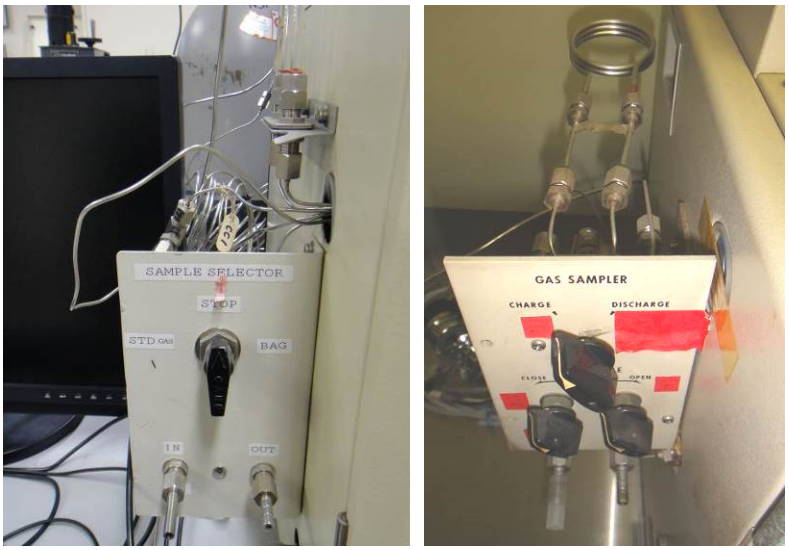

Figure 5.4. A gas sample loop and a manually switched injection valve.

Figure 5.5. Examples of automated gas injection systems for glass vials.

Figure 5.6. An example of automated gas injection system for plastic bags.

5.4. Standard gases

The output of a GC is the peak area or height of each separated gas. The peak area must be converted to concentration with reference to a known concentration of the target gas (i.e., a standard gas). We recommend using certified standard gases to calibrate GCs. It is also encouraged to cross-check the concentration between gas cylinders. Because the condition of a GC changes from day to day, it should be calibrated before every analysis.

Calibration at two concentration levels is adequate for FID-GC and ECD-GC when analyzing gas concentrations obtained by chamber measurement (i.e., at around the atmospheric concentration). The two calibration points of the standard gases should be outside the expected observed range (Figure 5.6). For example, suitable CH_4 standard gas concentrations are ~1.8 ppm (ambient level) and ~50 or 100 ppm (above the maximum level expected in the chamber).

It is sometimes difficult to obtain standard gases. If that is the case, it may be necessary to use working standard gases or to dilute a high-concentration standard gas. See Chapter 5.6 for further information.

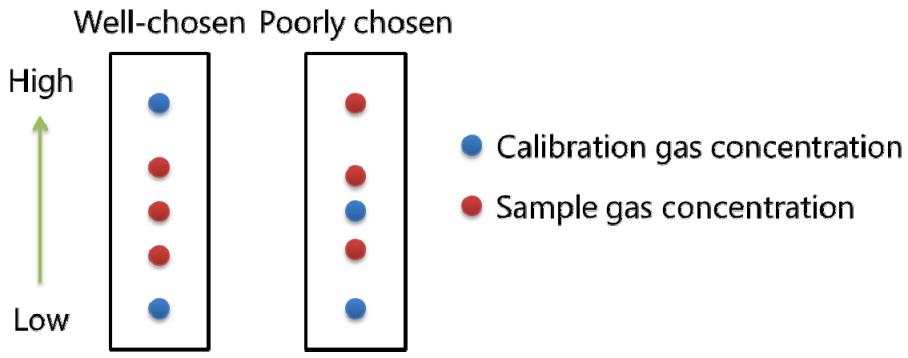

Figure 5.6. Examples of well-chosen and poorly chosen standard gas concentrations.

5.5. GC repeatability

5.5.1. Causes of errors

Multiple injections of even the same gas volume often give different peak areas. The repeatability (precision) of the analysis is increased by minimizing this error by improving GC settings and operation (see Chapters 5.2 and 5.3). However, it must be understood that some error is more or less inevitable due to the cumulative effect of the following errors.

First, the condition of a GC changes from moment to moment because of, for example, changes in column purity and the gas flow rate and purity. It is usually not possible to fix such instabilities on the spot, but they can be reduced by continuous maintenance and improving the GC settings.

Second, manual collection of the standard gas from the gas cylinder affects the trueness. We usually use a syringe, a plastic bag, or an evacuated vial to subdivide and temporarily store the standard gas, but this handling may cause contamination with ambient air. This error can be reduced by consistently collecting the standard gas with the same instruments each time.

Finally, during manual injection of the standard gas with a glass syringe, the gas may become contaminated with ambient air (affecting trueness). In addition to using appropriate methods and instruments (see Chapter 5.4), we should inject the gas at a constant stroke speed and push the start button of the GC after a fixed time (to increase precision). Thus, information on the GC performance and on the skill of manual operations is useful for GC maintenance and improvement.

5.5.2. Limit of detection and limit of quantification in GC analyses

The accuracy of an instrumental analysis is commonly represented by the limit of detection (LOD) and the limit of quantification (LOQ). The LOD is the lowest detectable quantity, whereas the LOQ is the lowest quantifiable quantity. Generally, the LOD is defined as 3 × standard deviation (σ) of repeated blank tests, and the LOQ is defined as 10σ. It should be noted, however, that many definitions of the detection limit have been proposed.

Here we apply the concepts of LOD and LOQ to GC analyses targeting GHG emissions from a rice paddy. LOD for GC analysis (LOD_{gc}) denotes the lowest detectable difference between the target gas concentration in a sample and its concentration in the ambient air. LOD_{gc} is defined as 3σ of repeated analysis of ambient air (standard gas). Similarly, LOQ for GC analysis (LOQ_{gc}) is the lowest quantifiable difference between the target gas concentration in a sample and its concentration in the ambient air. LOQ_{gc} is defined as 10σ of repeated analyses of ambient air (standard gas).

To determine LOD_{gc} and LOQ_{gc}, the gas analysis should be repeated 10–20 times in the same way. Ambient air (standard gas) samples should be stored in the same gas containers that are usually used for gas sampling. LOQ_{gc} is then used to calculate LOQ for gas flux

measurement (see Chapter 6.3). Table 5.2 shows two examples of the calculation of LOD_{gc} and LOQ_{gc}. In case 1, a CH_4 concentration of >2.79 ppm (i.e., mean 1.79 + LOQ_{gc} 1.00) is considered quantifiable when compared with ambient air, whereas in case 2, a concentration of >2.20 ppm (i.e., mean 2.00 + LOQ_{gc} 0.20) is quantifiable when compared with ambient air. These examples show that GC repeatability greatly affects LOD_{gc} and LOQ_{gc}.

Table 5.2. Template for calculating LOD_{gc} and LOQ_{gc} of CH_4 concentration analysis (ppm)

Replication number	Case 1	Case 2	Function in MS-Excel
1	1.89	1.97	
2	1.89	1.95	
3	1.93	2.01	
4	1.79	2.01	
5	1.70	2.00	
6	1.83	1.99	
7	1.66	2.01	
8	1.75	2.01	
9	1.71	2.05	
10	1.60	1.98	
11	1.75	2.00	
12	1.90	1.99	
13	1.88	2.01	
14	1.82	2.03	
15	1.79	2.01	
Mean	1.79	2.00	=average(#1:#15)
SD	0.10	0.02	=stdev.s(#1:#15)
CV (%)	5.59	1.00	=SD/Mean*100
LOD_{gc}	0.30	0.06	=SD*3
LOQ_{gc}	1.00	0.20	=SD*10

5.6. Evolving issues

It may not be possible to obtain certified standard gases because of their cost, or because it takes too much time to import them from abroad by ship (air transportation is often prohibited). If evacuated glass vials are used, it may be possible to store the collected gas samples for a long time (up to 1–2 months). However, a chronic inability to obtain standard gases cannot be solved by long-term storage. Here we discuss two possible stopgap measures.

Gas dilution. If a cylinder of certified standard gas with a concentration higher than the target range is available (Figure 5.6), the standard gas can be diluted as necessary with an inert gas (He or N_2). For example, three plastic bags can be connected to a plastic syringe: one containing an inert gas, one containing the high-concentration standard gas, and an empty bag to receive the diluted standard gas. Then the syringe is used to inject the standard gas and the inert gas in the necessary ratio into the empty bag (Figure 5.7). Diluted gases should be used soon or temporarily stored in evacuated glass vials. However, the accuracy of the dilution depends greatly on manual skill and must be checked by GC analysis.

Figure 5.7. A gas dilution tool consisting of a plastic syringe and three plastic bags. Bag 1 contains an inert gas; bag 2 contains a high-concentration standard gas; and bag 3 receives the diluted gas (low-concentration standard gas).

Working standard gas. If no certified standard gas is available, temporarily or chronically, air can be used as a working standard gas. However, this method requires a large-volume gas canister. The ambient air should be introduced into the canister in a pressurized state. The GHG concentrations in the gas in the canister should be analyzed at some later time by using certified standard gases. Evacuated glass vials should be used for long-term storage of the working standard gas.

6. Data processing

6.1. Introduction

Data processing involves (1) the calculation of the hourly gas flux from the analyzed gas concentrations over time in the chamber and (2) the calculation of seasonal or annual cumulative gas emissions from the calculated hourly gas flux. The common method for the flux calculation adopted by most researches may lead to under- or overestimation of the flux in some cases. This chapter explains how to accurately calculate hourly gas fluxes and total emissions from the gas concentrations determined by GC.

6.2. Calculation of hourly gas fluxes and cumulative emissions

6.2.1. Hourly gas flux

Linear regression is the recommended method for calculating the hourly CH_4 flux from a rice paddy. This method is based on the principle that the concentration gradient of CH_4 between flooded soil and the atmosphere is quite large, so that CH_4 can be considered to be emitted at a constant rate (Figure 6.1). The hourly fluxes of CH_4 (mg CH_4 m^{-2} h^{-1}) and N_2O (μg N m^{-2} h^{-1}) are calculated as follows:

$$Flux_{CH4} = \frac{\Delta C}{\Delta t} \times \frac{V}{A} \times \rho \times \frac{273}{273+T}$$

$$Flux_{N2O} = \frac{\Delta C}{\Delta t} \times \frac{V}{A} \times \rho \times \frac{273}{273+T} \times \frac{28}{44}$$

where ΔC/Δt is the concentration change over time (ppm-CH_4 or ppb-N_2O h^{-1}); V is chamber volume (m^3); A is chamber area (footprint; m^2); ρ is gas density (0.717 kg m^{-3} for CH_4 and 1.977 kg m^{-3} for N_2O at 0°C); and T is the mean air temperature inside the chamber (°C).

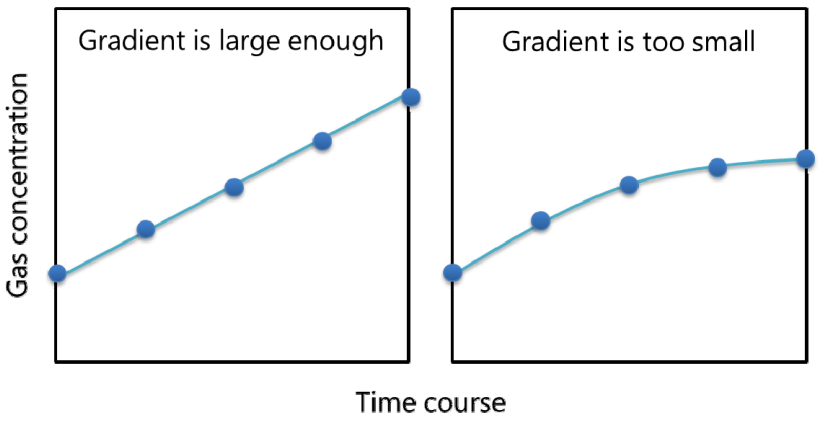

Figure 6.1. Temporal changes in the gas concentration inside the chamber, showing a large concentration gradient between the soil and the atmosphere (left) and one that is too small (right).

The assumption of a large concentration gradient is often not justified in the case of the N_2O flux from a non-flooded soil. In an upland field, long-term chamber placement can cause saturation of the N_2O concentration inside the chamber (Parkin and Venterea, 2010) (Figure 6.1). Therefore, the pattern of observed concentration increases should be checked under every situation and for every gas to determine the appropriate method. For other calculation methods, see Parkin and Venterea (2010) and Venterea et al. (2012).

Linear regression is not always the best method, and it may be theoretically inappropriate, for calculating the CH_4 flux from a flooded soil. For example, the rate of change in the concentration might change at mid-morning, or an unpredictable natural CH_4 ebullition may occur during chamber deployment, causing the linearity to be poor (Figure 6.2). In such cases, we can use a concentration difference method (i.e., two-point linear regression using the concentrations of the initial and last gas samples) instead. For instance, in the case of CH_4 ebullition, using the inappropriate calculation method can cause a considerable error (11%) (Table 6.1). It should be noted that adoption of the concentration difference method is appropriate on the assumption that the measurements were conducted properly. Errors from human-induced CH_4 ebullition, for example, cannot be avoided by using this alternative method (see Chapter 4.8.2). In addition, when the original number of gas samples per chamber deployment is three, the calculated flux becomes the same between the linear regression and the concertation difference method.

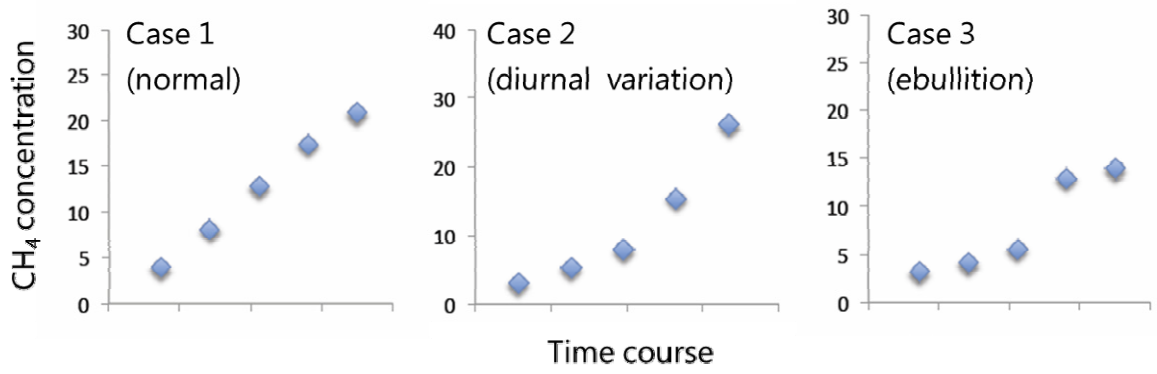

Figure 6.2. Examples of three observed patterns of CH_4 concentration changes over time.

Table 6.1. CH_4 fluxes calculated by two methods for each of the three cases shown in Figure 6.2

Variables	Case 1	Case 2	Case 3
Flux calculated from linear regression (A, mg CH_4 m^{-2} h^{-1})	15.97	13.62	11.68
Adjusted R^2	1.00	0.86	0.85
p value	<0.001	0.014	0.017
Flux calculated from the concentration difference (B, mg CH_4 m^{-2} h^{-1})	15.63	13.97	10.39
B/A	0.98	1.03	0.89

6.2.2. Significance of linear regression

One criterion used to check whether the number of gas samples collected during chamber deployment is adequate is the statistical significance of the linear regression of gas concentration over time. The use of this criterion is based on the assumption that the gas is emitted at a constant rate (via diffusion through the rice plants, not via ebullition). To analyze the significance of the regression slope, at least three samples are needed. However, the coefficient of determination (R^2) must be very high to detect significance with three samples per chamber deployment (Table 6.2). Therefore, as discussed in Chapter 4.5, we recommend collecting at least three samples during a 20–30-min chamber deployment (see also Chapter 4.8.2). In this case, it is possible to disregard outliers of the gas concentration data based on experience and judgment of past performance of the site, instrument function, or chamber efficacy (Parkin and Venterea, 2010).

Although some researchers use the statistical significance of the regression slope to decide whether the calculated flux is acceptable or not (i.e., considered as zero or other fixed value), the relationship can be non-significant for various reasons, as explained in Chapter 6.2.1. Natural or human-induced ebullition can cause the regression to be non-significant even when the gas flux is high. If ebullition occurs naturally, the rejection of a non-significant flux can cause the data to be misinterpreted. Similarly, a very low gas flux can lead to non-significance, especially when the GC performance is poor (see Chapter 5.5). For ways to identify low but meaningful fluxes, see Chapter 6.3. Thus, the reason for non-linearity should be identified before statistical significance is used as the criterion for determining whether the number of gas samples is adequate and whether the calculated flux is acceptable.

Table 6.2. Values of the coefficient of determination (R^2) needed to detect significance ($p < 0.05$ or $p < 0.1$) for different numbers of gas samples in a linear correlation

Significance level	3 samples	4 samples	5 samples	6 samples
$p < 0.05$	0.994	0.903	0.773	0.659
$p < 0.1$	0.976	0.810	0.650	0.533

6.2.3. Cumulative gas emission

Use of a trapezoidal integration method (i.e., linear interpolation and numerical integration between sampling times) is recommended for calculating cumulative gas emissions. There are three steps to this calculation (Figure 6.3). First, calculate the daily gas flux by multiplying the daily mean hourly gas flux by 24. Second, calculate the emission between every two consecutive measurements using the trapezoidal rule. Third, sum up the areas of all the trapezoids. It should be noted that low-frequency (i.e., long interval) flux measurements during agricultural management events (e.g., drainage and N topdressing) may cause under- or overestimation of the cumulative emissions (see Chapter 6.4.2).

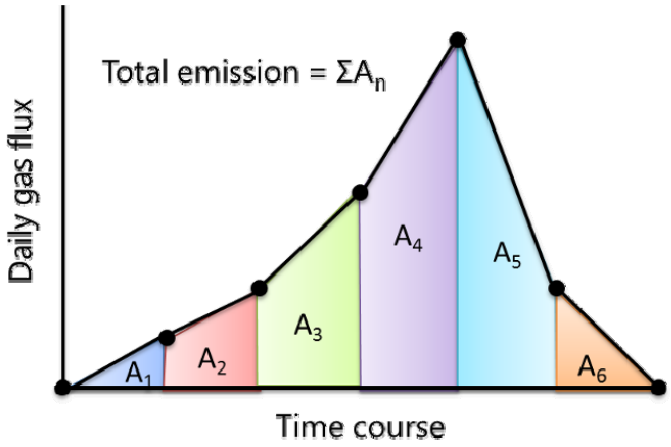

Figure 6.3. A schematic diagram of the trapezoidal integration method.

6.3. Limit of quantification for the gas flux

6.3.1. Introduction

Systematic and random errors associated with gas flux measurements vary across laboratories, because the errors depend on relative instrumental performance, the analysts who perform the analyses, and the methods used. For (1) standardized reporting of a MRV (measurement, reporting, and verification) project and (2) comparative assessment of multiple locations, it is essential to assess the analytical uncertainty associated with all sources of variability in the observed data.

In Chapter 5.5.3, we introduced the concepts of LOD and LOQ and applied them to GC analysis of GHG emissions from a rice paddy (LOD_{gc} and LOQ_{gc}). Here we further apply these concepts to the gas flux calculation. Our proposed method for estimating LOD and LOQ of the gas flux (LOD_{flux} and LOQ_{flux}) quantifies the overall precision and sensitivity of the methods used, from gas sampling to GC analysis. That is, we accept the measured gas flux as a significant value if the difference in the measured gas concentration between the initial sample and the last sample during the chamber deployment is higher than the LOQ_{gc}. However, it should be noted that human-caused errors, such as CH_4 ebullition and use of a non-airtight chamber, are not captured by the LOQ_{flux} calculation.

6.3.2. Detailed procedure

LOQ_{flux} of CH_4 (mg CH_4 m^{-2} h^{-1}) is calculated as follows:

$$LOQ_{flux} = LOQ_{gc} \times \frac{V}{A} \times \rho \times \frac{273}{273+T} \times \frac{60}{t}$$

where V is chamber volume (m^3); A is chamber area (m^2); ρ is gas density (0.717 kg m^{-3} at 0°C); T is the mean air temperature inside the chamber (°C); and t is the chamber deployment time (min).

This equation is almost the same as that for the flux calculation (see Chapter 6.2.1), and it shows that a shorter deployment time and/or a higher chamber height (V/A) increases LOQ_{flux} (Table 6.3). Furthermore, a more accurate GC analysis (LOQ_{gc}) is obviously important for lowering LOQ_{flux}.

At present there is no single way to handle data below the LOQ_{flux} in a scientific paper. Parkin et al. (2012) suggested three possible ways to manage flux data below a detection limit (here LOQ_{flux}). First, the data can be presented "as is" (without any correction); second, they can be presented with a disclaimer; third, they can be set to zero. Whichever way is chosen should be indicated in the methods section of a scientific paper. LOQ_{gc} and LOQ_{flux} should be determined at least once every cropping season to check the overall method performance.

Table 6.3. Examples of calculating LOD_{flux} and LOQ_{flux} under different conditions

	Case 1	Case 2	Case 3	Case 4
LOD_{gc} (ppm)	0.30	0.06	0.06	0.06
LOQ_{gc} (ppm)	1.00	0.20	0.20	0.20
LOD_{flux} (mg CH_4 m^{-2} h^{-1})	0.39	0.08	0.12	0.14
LOQ_{flux} (mg CH_4 m^{-2} h^{-1})	1.31	0.26	0.39	0.47
V (m^3)	0.18	0.18	0.18	0.22
A (m^2)	0.18	0.18	0.18	0.18
ρ (kg m^{-3})	0.717	0.717	0.717	0.717
T (°C)	25	25	25	25
t (min)	30	30	20	20

Gray shading highlights large differences between two adjacent cells.

6.4. Evolving issues

6.4.1. Correction for inadequate chamber area

As described in Chapter 3.3, the chamber cross-sectional area (its footprint) should generally be a multiple of plant density for transplanted fields. This simplifies determination of the unit area when calculating the gas flux. If the chamber area is not a multiple of plant density, a correction needs to be applied in the flux calculation. Because the emission pathway differs between CH_4 and N_2O, and between flooded and drained periods, the unit-area correction used for each is based on different assumptions.

For CH_4 flux in a flooded field, a chamber that is the wrong size is not a critical problem, because most CH_4 is emitted via the rice plants (Figure 6.4). For example, if a chamber with a 30 cm × 30 cm footprint covers four plants, each occupying an area of 20 cm × 20 cm (i.e.,

total area of 40 cm × 40 cm), the area covered by the chamber can be assumed to be 40 cm × 40 cm when calculating the area-based gas flux. On the other hand, for the CH_4 flux in a drained field and the N_2O flux in flooded or drained fields, the gases are emitted directly (exchanged) from the drained soil or the surface water to the atmosphere (Figure 6.4). Therefore, the original chamber footprint (30 cm × 30 cm in the above example) should be used when calculating the area-based gas flux.

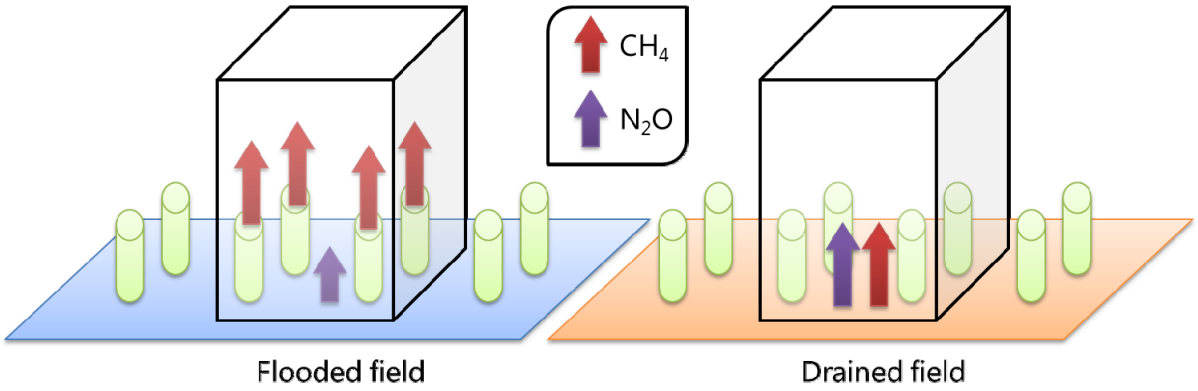

Figure 6.4. Main emission pathways of CH_4 and N_2O during flooded and drained periods.

6.4.2. Correction for a missing flux peak

As described in Chapter 4.4, we recommend measuring gas fluxes just before agricultural management events and then frequently until the flux peak has passed. Lack of gas flux data from just before and during temporary drainage and N fertilization events may cause considerable over- or underestimation of the cumulative emissions (Figure 6.5). Such gaps in the measurements should be recorded. At least in the case of CH_4 and N_2O fluxes just before the drainage and N_2O flux just before N topdressing, the flux levels are not likely to differ drastically from the preceding measurement. In such cases, it can be assumed that the gas flux just (1 day) before the agricultural management event was the same as the one just preceding it.

Figure 6.5. Examples of the consequences of inadequate gas sampling before and during agricultural management events.

6.4.3. The significance of linear regression and/or LOQ_{flux}

By itself, the significance of the linear regression (see Chapter 6.2.2) is not a sufficient criterion for determining whether the measured gas flux is acceptable. Because the significance is strongly affected by the LOQ_{gc} (see Chapter 5.5), it is difficult to tell whether the non-significance is due to poor GC performance or to human error when the measured gas concentration is near the ambient level. Therefore, when the measured gas concentration is near the ambient level, we recommend using both the significance of the regression and LOQ_{flux} as criteria. That is, if the measured gas flux fails to pass either criterion, it should be set to zero.

Non-significance accompanying high positive or negative fluxes indicates human error or natural CH_4 ebullition, because GC performance is better at a high concentration range. Human error should be avoided by careful sample collection and adherence to GC protocols. However, if such error cannot be avoided, we recommend prioritizing LOQ_{flux} as the criterion for determining the acceptability of measured fluxes (i.e., ignore the linear regression result). As described in Chapter 6.2.1, the concentration difference method can be used for the flux calculation in such cases. If a high but non-significant gas flux is regarded as zero or other fixed value, the cumulative emissions are likely to be misestimated.

7. Auxiliary measurements

7.1. Introduction

Auxiliary data are essential to provide supporting evidence for the interpretation and generalization (modeling) of observed GHG emissions from a rice paddy. These data generally include rice productivity, soil and water environment, and weather conditions. Some of these data are commonly collected in agricultural studies, but others are specific to GHG studies, thereby requiring additional research instruments and skills. Provision of field metadata (i.e., data about field data) is helpful for secondary users of the field data (i.e., for meta-analyses, model simulations, etc.). This chapter introduces recommended and optional measurements.

7.2. Experimental conditions

Table 7.1 lists basic information that should be collected for field GHG studies. Weather data can be recorded by an automated meteorological monitoring system throughout the year. Nearby weather station data can also be used. Weekly weather data is not adequate for full interpretation of the measured GHG fluxes. Daily solar radiation and/or hours of sunshine are useful for model simulations.

The type of water supply (i.e., rice ecosystem type) may be, for example, irrigated, flood-prone rainfed, or dry-prone rainfed. Soil C and N contents are related to soil C sequestration, another option for the mitigation of GHG emissions from agricultural soils (see Chapter 7.5.3). In addition to common soil properties, we recommend collecting information on field drainage conditions, especially if water management is the research subject.

Table 7.1. Examples of basic information on field location, weather, and the soil and water environment

Category	Item
Location	Country, province/state, (nearest) city, site name, latitude/longitude, *topography (elevation)*
Weather	Climate zone, wet and dry seasons, precipitation, air temperature, *daily solar radiation*, *sunshine hours*
Water	Type of water supply, *groundwater level*, *water percolation rate (hydraulic conductivity)*
Soil	Soil taxonomy (World Reference Base for Soil Resources and/or USDA Soil Taxonomy), total C and N contents, plow layer depth, bulk density, texture (sand, silt, clay), *free iron content*

Italics indicate optional items.

7.3. Agricultural management practices

Table 7.2 lists information related to agricultural management practices that should be recorded. The field cultivation history covering at least the three preceding years should be collected. This is particularly important for CH_4 emissions if non-rice crops have been cultivated (paddy-upland rotation; Nishimura et al., 2011). In addition, information about all agricultural practices used throughout the year should be recorded. Details of water management during the rice growing period are especially important because water management drastically alters soil redox conditions. Therefore, the surface water depth should be measured frequently manually (Figure 7.1) or automatically (Figure 7.2) to ensure that the stated practice is being adhered to.

Table 7.2. Examples of information related to basic agricultural practices

Category	Item
Cultivation history	Name of crop(s), number of crops per year, organic amendments (type and rate), soil water status in fallow
Current practices	Date/duration, method/type, or rate/amount of each
Water management	Surface water level, *water intake*, *surface water drainage*, *pipe/tile drainage*

Italics indicate optional items.

Figure 7.1. PVC pipes with multiple holes on the sidewall that installed in a paddy soil to monitor the surface water depth.

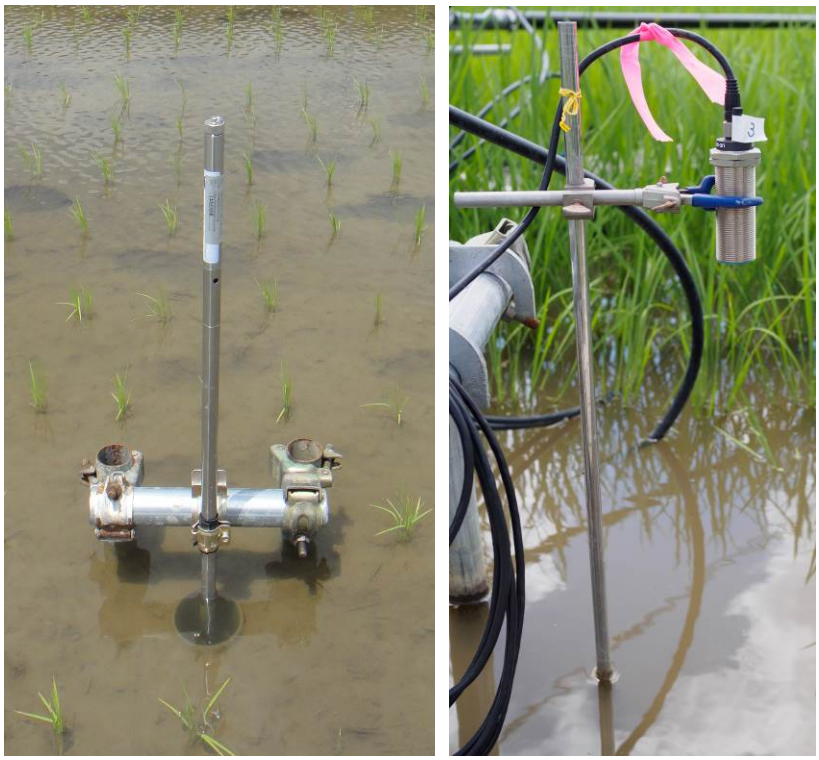

Figure 7.2. Automated sensors for surface water level: left, capacitance type; right, ultrasonic type.

7.4. Rice growth and yield

Record the denomination of rice variety. Periodically (e.g., biweekly) (1) plant height, (2) the number of tillers/ears per unit area or per hill, (3) aboveground biomass, and (4) root biomass (optional) should be measured. In addition, records of the rice growth stages, especially the date of heading, are useful for interpreting the seasonal pattern of CH_4 fluxes (Minamikawa et al., 2012). Disease and insect damage to rice should be observed and recorded. Yields of grain and straw should be measured by a quadrate sampling method. These enable calculation of yield-scaled GHG emissions (i.e., GHG intensity), which has recently been a topic of interest (e.g., Linquist et al., 2012). In addition, rice growth and yield inside and outside of the chamber should be compared to evaluate the so-called chamber effect (Nishimura et al., 2004). Yield component analysis data are useful for further investigations.

7.5. Specific measurements

7.5.1. Soil redox chemistry

Sequential reduction of soil oxidants after flooding controls paddy soil biogeochemistry. Redox potential is a useful measure of reductive conditions in a flooded paddy soil. To measure soil redox potential, platinum-tipped electrodes and a portable meter with a reference electrode are usually used (Figure 7.3). Before using the equipment on-site, the

output of each electrode should be checked by using a standard chemical (e.g., quinhydrone), because there can be substantial differences in output among electrodes. Data are generally collected at a soil depth of 5 cm. Props can be used to support the electrode and minimize shaking when it is being connected to the meter. Because of the high spatial variability of soil redox status, at least three electrodes should be used per plot. It should be noted that drainage events can cause the soil to crack and expose the platinum tip of the electrode to water or air.

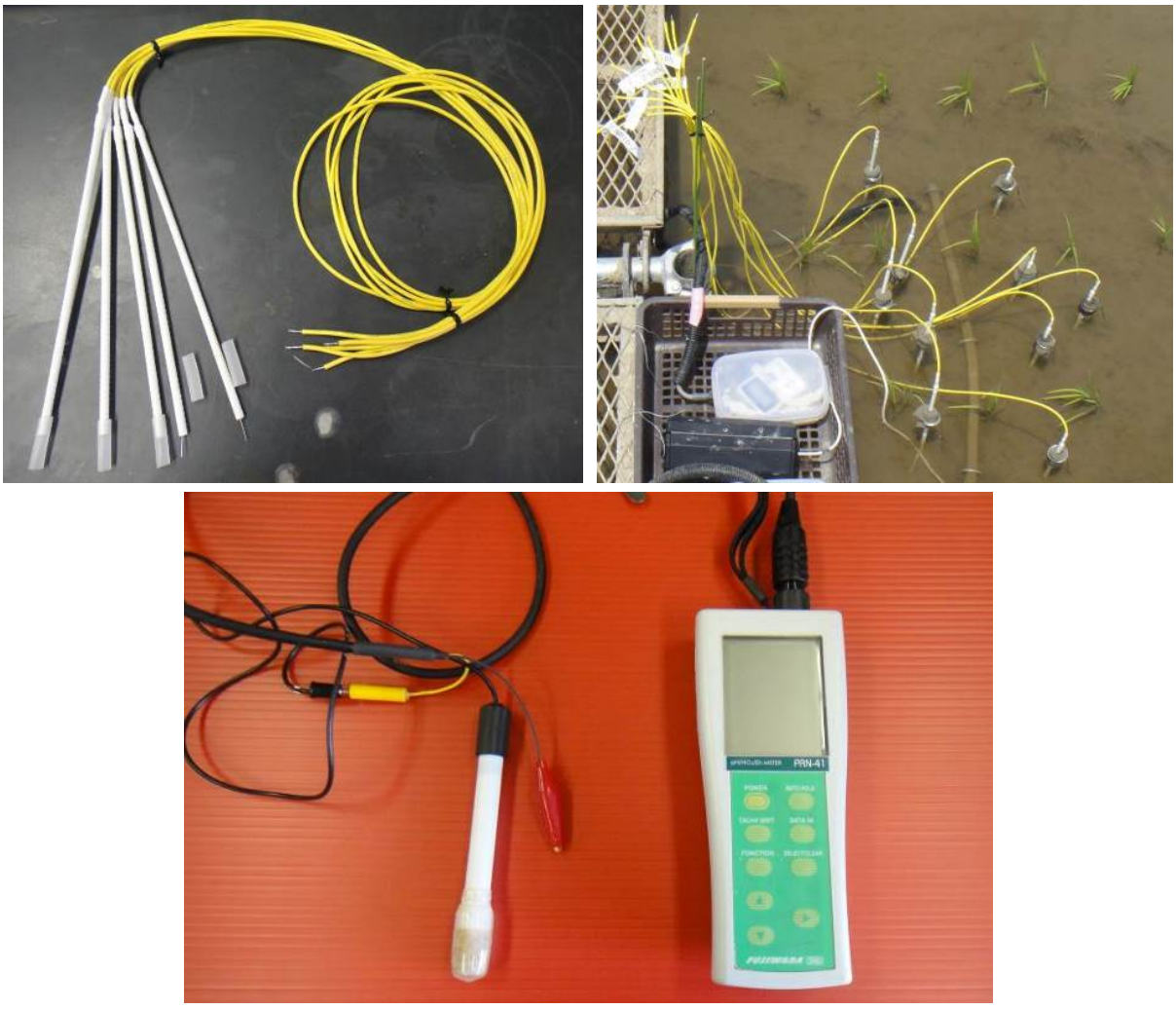

Figure 7.3. Platinum-tipped electrodes (left), electrodes installed in a flooded paddy soil (right), and a portable redox potential meter (bottom).

Iron is the dominant redox substance in a paddy soil. After a field is flooded, soil ferrous iron (Fe(II), a reduced form of iron) increases and reaches a plateau. Although the biologically reducible soil Fe(II) can be regressed from the empirical equation with various forms of Fe (Cheng et al., 2007; Smakgahn et al., 2009), soil Fe(II) can be determined by colorimetry using *o*-phenanthroline. See Tokida et al. (2010) for the detailed method.

7.5.2. Soil temperature and moisture

Soil temperature can be recorded by inserting a thermometer to a depth of 5 cm in flooded or drained soil. The soil moisture content under the non-flooded condition is a driving factor of the N_2O flux. The measurement depth should be the same as for soil temperature. As mentioned in Chapter 7.2, we recommend monitoring both at 1-hour intervals throughout the year by using automated sensors/loggers. However, if automated instruments are not available, manual monitoring each time the gas flux is measured still provides useful information.

7.5.3. Soil C and N contents

Soil C sequestration is one of the most effective options for mitigating GHG emissions from agricultural soils. IPCC (2006) recommends investigating soil C storage down to 30 cm depth. However, because soil thickness is changed by agricultural practices and natural events, soil mass equivalent (Ellert and Bettany, 1995) can be used for offsetting their effects. The product of the soil bulk density and the soil CN content is the CN storage. We recommend measuring them at various depths (e.g., 2.5–7.5 cm for 0–10 cm layer, 12.5–17.5 cm for 10–20 cm layer, and 22.5–27.5 cm for 20–30 cm layer) (Figure 7.4). A long-term (i.e., several years) investigation is required to accurately evaluate storage changes.

N substrates for soil N_2O production include ammonium and nitrate. Frequent measurement of soil inorganic N contents is useful for interpreting the seasonal N_2O flux pattern. Soil extracted with KCl solution can be analyzed for ammonium and nitrate concentrations by using, for example, a continuous flow analyzer.

Figure 7.4. Stainless coring tubes (100 mL, left) and soil collection using a coring tube (right).

References

Adhya, T.K., Rath, A.K., Gupta, P.K., Rao, V.R., Das, S.N., Parida, K.M., Parashar, D.C., Sethunathan, N. (1994) Methane emission from flooded rice fields under irrigated conditions. *Biology and Fertility of Soils*, 18, 245-248.

Adviento-Borbe, M.A., Padilla, G.N., Pittelkow, C.M., Simmonds, M., van Kessel, C., Linquist, B. (2015) Methane and nitrous oxide emissions from flooded rice systems following the end-of-season drain. *Journal of Environmental Quality*, 44, 1071–1079

Aulakh, M.S., Wassmann, R., Rennenberg, H. (2001) Methane emissions from rice fields - quantification, mechanisms, role of management, and mitigation options. *Advances in Agronomy*, 70, 193-260.

Bain, W.G., Hutrya, L., Patterson, D.C., Bright, A.V., Daube, B.C., Munger, J.W., Wofsy, S.C. (2005) Wind-induced error in the measurement of soil respiration using closed dynamic chambers. *Agricultural and Forest Meteorology*, 131, 225-232.

Balashova, V.V., Zavarzin, G.A. (1980) Anaerobic reduction of ferric iron by hydrogen bacteria. *Microbiology*, 48, 635-639.

Banker, B.C., Kludze, H.K., Alford, D.P., Delaune, R.D., Lindau, C.W. (1995) Methane sources and sinks in paddy rice soils: Relationship to emissions. *Agriculture, Ecosystems and Environment*, 53, 243-251.

Blanco, G., Gerlagh, R., Suh, S. et al. (2014) Drivers, trends and mitigation. In: Edenhofer, O., Pichs-Madruga, R., Sokona, Y. et al. (eds.) Climate change 2014: Mitigation of climate change. Contribution of working group III to the fifth assessment report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom and New York, NY, USA, Cambridge University Press.

Buendia, L.V., Neue, H.U., Wassmann, R., Lantin, R.S., Javellana, A. M., Arah, J., Wang, Z., Wanfang, L., Makarim, A.K., Corton, T.M., Charoensilp, N. (1998) An efficient sampling strategy for estimating methane emission from rice field. *Chemosphere*, 36, 395-407.

Butterbach-Bahl, K., Papen, H., Rennenberg, H. (1997) Impact of gas transport through rice cultivars on methane emission from rice paddy fields. *Plant, Cell and Environment*, 20, 1175-1183.

Byrnes, B.H., Austin, E.R., Tays, B.K. (1995) Methane emissions from flooded rice soils and plants under controlled conditions. *Soil Biology and Biochemistry*, 27, 331-339.

Cheng, W., Yagi, K., Akiyama, H., Nishimura, S., Sudo, S., Fumoto, T., Hasegawa, T., Hartley, A.E., Megonigal, J.P. (2007) An empirical model of soil chemical properties that regulate methane production in Japanese rice paddy soils. *Journal of Environmental Quality*, 36, 1920-1925.

Ciais, P., Sabine, C., Bala, G. et al. (2013) Carbon and other biogeochemical cycles. In: Stocker, T.F., Qin, D., Plattner, G.-K. et al. (eds) Climate change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom and New York, NY, USA, Cambridge University Press.

Cicerone, R.J., Shetter, J.D. (1981) Sources of atmospheric methane: Measurements in rice paddies and a discussion. *Journal of Geophysical Research*, 86, 7203-7209.

Clever, H.L., Young, C.L. (1987) Methane. In: IUPAC solubility data series. Oxford, Pergamon.

Clough, T.J., P. Rochette, P., Thomas, S.M., Pihlatie, M., Christiansen, J.R., Thorman, R.E. (2012) 2. Chamber design. In: de Klein, C., Harvey, M. (eds.) Nitrous Oxide Chamber Methodology Guidelines. Ministry for Primary Industries, Wellington, New Zealand, pp. 19-33.

Conrad, R. (2002) Control of microbial methane production in wetland rice fields. *Nutrient Cycling in Agroecosystems*, 64, 59-69.

Dannenberg, S., Conrad, R. (1999) Effect of rice plants on methane production and rhizospheric metabolism in paddy soil. *Biogeochemistry*, 45, 53-71.

Denier van der Gon, H.A.C., van Breemen, N. (1993) Diffusion-controlled transport of methane from soil to atmosphere as mediated by rice plants. *Biogeochemistry*, 21, 177-190.

Ellert, B.H., Bettany, J.R. (1995) Calculation of organic matter and nutrients stored in soils under contrasting management regimes. *Canadian Journal of Soil Science*, 75, 529-538.

Eusufzai, M.K., Tokida, T., Okada, M., Sugiyama, S., Liu, G.C., Nakajima, M., Sameshima, R. (2010) Methane emission from rice fields as affected by land use change. *Agriculture, Ecosystems and Environment*, 139, 742-748.

Fisher, R.A. (1926) The arrangement of field experiments. *Journal of the Ministry of Agriculture of Great Britain*, 33, 503-513.

Frenzel, P., Rothfuss, F., Conrad, R. (1992) Oxygen profiles and methane turnover in a flooded rice microcosm. *Biology and Fertility of Soils*, 14, 84-89.

GRISP (2013) Rice almanac, 4th edition, Los Baños, Philippines, International Rice Research Institute.

Hayashi, K., Tokida, T., Kajiura, M., Yanai, Y., Yano, M. (2015) Cropland soil–plant systems control production and consumption of methane and nitrous oxide and their emissions to the atmosphere. *Soil Science and Plant Nutrition*, 61, 2-33.

Himmelblau, D.M. (1964) Diffusion of dissolved gases in liquids. *Chemical Reviews*, 64, 527-550.

Holzapfel-Pschorn, A., Conrad, R., Seiler, W. (1986) Effects of vegetation on the emission of methane from submerged paddy soil. *Plant and Soil*, 92, 223-233.

Holzapfel-Pschorn, A., Seiler, W. (1986) Methane emission during a cultivation period from an Italian rice paddy. *Journal of Geophysical Research*, 91, 11803-11814.

Hou, A.X., Chen, G.X., Wang, Z.P., Van Cleemput, O., Patrick, Jr., W.H. (2000) Methane and Nitrous Oxide Emissions from a Rice Field in Relation to Soil Redox and Microbiological Processes. *Soil Science Society of America Journal*. 64, 2180-2186.

Hurlbert, S.H. (1984) Pseudoreplication and the Design of Ecological Field Experiments. *Ecological Monographs*, 54, 187-211.

Hutchinson, G.L., Mosier, A.R. (1981) Improved soil cover method for field measurement of nitrous oxide fluxes. *Soil Science Society of America Journal*, 45, 311-316.

IAEA (1992) Manual on measurement of methane and nitrous oxide emissions from agriculture. IAEA, Vienna, Austria.

IGAC (1994) Global Measurement Standardization of Methane Emissions from Irrigated Rice Cultivation: A Report of the Rice Cultivation and Trace Gas Exchange Activity (RICE) of the International Global Atmospheric Chemistry (IGAC) Project. IGAC Core Project Office, Massachusetts Institute of Technology, Cambridge, USA.

IPCC (2006) Volume 4. Agriculture, Forestry and Other Land Use: 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Eggleston, H.S., Buendia, L., Miwa, K., Ngara, T., Tanabe, K. (eds.). IGES, Hayama.

Kamura, T., Takai, Y., Ishikawa, K. (1963) Microbial reduction mechanism of ferric iron in paddy soils (part I). *Soil Science and Plant Nutrition*, 9, 171-175.

Katayanagi, N., Tokida, T. (in preparation) On the accuracy of chamber-based methane emission measurements: How many samples are necessary per chamber deployment?

Kato, S., Watanabe, K. (2010) Ecological and evolutionary interactions in syntrophic methanogenic consortia. *Microbes and Environments*, 25, 145-151.

Khalil, M.A.K., Butenhoff, C.L. (2008) Spatial variability of methane emissions from rice fields and implications for experimental design. *Journal of Geophysical Research*, 113, G00A09.

Kimura, M., Murase, J., Lu, Y. (2004) Carbon cycling in rice field ecosystems in the context of input, decomposition and translocation of organic materials and the fates of their end products (CO_2 and CH_4). *Soil Biology and Biochemistry*, 36, 1399-1416.

Linquist, B., Van Groenigen, K.J., Adviento-Borbe, M.A., Pittelkow, C., Van Kessel, C. (2012) An agronomic assessment of greenhouse gas emissions from major cereal crops. *Global Change Biology*, 18, 194-209.

Lovley, D.R., Phillips, E.J.P. (1988) Novel mode of microbial energy metabolism: Organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Applied and Environmental Microbiology*, 54, 1472-1480.

Minamikawa, K., Yagi, K., Tokida, T., Sander, B.O., Wassmann, R. (2012) Appropriate frequency and time of day to measure methane emissions from an irrigated rice paddy in Japan using the manual closed chamber method. *Greenhouse Gas Measurement and Management*, 2, 118-128.

Myhre, G., Shindell, D., Bréon, F.-M. et al. (2013) Anthropogenic and natural radiative forcing. In: Stocker, T.F., Qin, D., Plattner, G.-K. et al. (eds.) Climate change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom and New York, NY, USA, Cambridge University Press.

Nishimura, S., Sawamoto, T., Akiyama, H., Sudo, S., Yagi, K., (2004) Methane and nitrous oxide emissions from a paddy field with Japanese conventional water management and fertilizer application. *Global Biogeochemical Cycles*, 18, GB2017.

Nishimura, S., Akiyama, H., Sudo, S., Fumoto, T., Cheng, W., Yagi, K. (2011) Combined emission of CH_4 and N_2O from a paddy field was reduced by preceding upland crop cultivation. *Soil Science and Plant Nutrition*, 57, 167-178.

Nouchi, I., Mariko, S., Aoki, K. (1990) Mechanism of methane transport from the rhizosphere to the atmosphere through rice plants. *Plant Physiology*, 94, 59-66.

Parkin T.B., Venterea, R.T. (2010) Chapter 3. Chamber-Based Trace Gas Flux Measurements. In: Follett, R.F. (ed.) Sampling Protocols. Pp. 3.1-3.39.

Parkin, T.B., Venterea, R.T., Hargreaves, S.K. (2012) Calculating the detection limits of chamber-based soil greenhouse gas flux measurements. *Journal of Environmental Quality*, 41, 705-715.

Rochette, P., Eriksen-Hamel, N.S. (2008) Chamber Measurements of Soil Nitrous Oxide Flux: Are Absolute Values Reliable? *Soil Science Society of America Journal*, 72, 331-342.

Rochette, P., Chadwick, D.R., de Klein, C.A.M., Cameron, K. (2012) 3. Deployment protocol. In: de Klein,

C., Harvey, M. (eds.) Nitrous Oxide Chamber Methodology Guidelines. Ministry for Primary Industries, Wellington, New Zealand, pp. 34-55.

Rothfuss, F., Conrad, R. (1998) Effect of gas bubbles on the diffusive flux of methane in anoxic paddy soil. *Limnology and Oceanography*, 43, 1511-1518.

Sander, B.O., Wassmann, R. (2014) Common practices for manual greenhouse gas sampling in rice production: a literature study on sampling modalities of the closed chamber method. *Greenhouse Gas Measurement and Management*, 4, 1-13.

Sass, R.L., Fisher Jr. F.M., Andrews, J.A. (2002) Spatial variability in methane emissions from a Texas rice field with some general implications. *Global Biogeochemical Cycles*, 16, 15.1–15.7.

Satpathy, S.N., Rath, A.K., Ramakrishnan, B., Rao, V.R., Adhya, T.K., Sethunathan, N. (1997) Diurnal variation in methane efflux at different growth stages of tropical rice. *Plant and Soil*, 195, 267-271.

Schütz, H., Seiler, W., Conrad, R. (1989) Processes involved in formation and emission of methane in rice paddies. *Biogeochemistry*, 7, 33-53.

Schink, B. (1997) Energetics of syntrophic cooperation in methanogenic degradation. *Microbiology and Molecular Biology Reviews*, 61, 262-280.

Smakgahn, K., Fumoto, T., Yagi, K. (2009) Validation of revised DNDC model for methane emissions from irrigated rice fields in Thailand and sensitivity analysis of key factors. *Journal of Geophysical Research*, 114, G02017

Smith, P., Bustamante, M., Ahammad, H. et al. (2014) Agriculture, forestry and other land use (AFOLU). In: Edenhofer, O., Pichs-Madruga, R., Sokona, Y. et al. (eds.) Climate change 2014: Mitigation of climate change. Contribution of working group III to the fifth assessment report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom and New York, NY, USA, Cambridge University Press.

Takai, Y. (1970) The mechanism of methane fermentation in flooded paddy soil. *Soil Science and Plant Nutrition*, 16, 238-244.

Takai, Y., Kamura, T. (1966) The mechanism of reduction in waterlogged paddy soil. *Folia Microbiologica*, 11, 304-313.

Tokida, T., Fumoto, T., Cheng, W. et al. (2010) Effects of free-air CO_2 enrichment (FACE) and soil warming on CH_4 emission from a rice paddy field: impact assessment and stoichiometric evaluation. *Biogeosciences*, 7, 2639-2653.

Tokida, T., Adachi, M., Cheng, W.G. et al. (2011) Methane and soil CO_2 production from current-season photosynthates in a rice paddy exposed to elevated CO_2 concentration and soil temperature. *Global Change Biology*, 17, 3327-3337.

Tokida, T., Cheng, W., Adachi, M., Matsunami, T., Nakamura, H., Okada, M., Hasegawa, T. (2013) The contribution of entrapped gas bubbles to the soil methane pool and their role in methane emission from rice paddy soil in free-air [CO_2] enrichment and soil warming experiments. *Plant and Soil*, 364, 131-143.

Uzaki, M., Mizutani, H., Wada, E. (1991) Carbon isotope composition of CH_4 from rice paddies in japan. *Biogeochemistry*, 13, 159-175.

Venterea, R.T., Parkin, T.B., Cardenas, L., Petersen, S.O., Pedersen, A.R. (2012) 6. Data analysis considerations. In: de Klein, C., Harvey, M. (eds.) Nitrous Oxide Chamber Methodology Guidelines. Ministry for Primary Industries, Wellington, New Zealand, pp. 95-121.

Wang, B., Neue, H.U., Samonte, H.P. (1997) Role of rice in mediating methane emission. *Plant and Soil*, 189, 107-115.

Wassmann, R., Neue, H.U., Alberto, M.C.R. et al. (1996) Fluxes and pools of methane in wetland rice soil with varying organic inputs. *Environmental Monitoring and Assessment*, 42, 163-173.

Wassmann, R., Buendia, L.V., Lantin, R.S., Bueno, C.S., Lubigan, L.A., Umali, A., Nocon, N.N., Javellana, A.M., Neue, H.U. (2000) Mechanisms of crop management impact on methane emissions from rice fields in Los Baños, Philippines. *Nutrient Cycling in Agroecosystems*, 58, 107-119.

Watanabe, A., Murase, J., Katoh, K., Kimura, M. (1994) Methane production and its fate in paddy fields. V. Fate of methane remaining in paddy soil at harvesting stage. *Soil Science and Plant Nutrition*, 40, 221-230.

Watanabe, A., Takeda, T., Kimura, M. (1999) Evaluation of origins of CH_4 carbon emitted from rice paddies. *Journal of Geophysical Research*, 104, 23623-23629.

Weller, S., Kraus, D., Ayag, K.R.P., Wassmann, R., Alberto, M.C.R., Butterbach-Bahl, K., Kiese, R. (2015) Methane and nitrous oxide emissions from rice and maize production in diversified rice cropping systems. *Nutrient Cycling in Agroecosystems*, 101, 37-53.

Wikipedia contributors. "Accuracy and precision." Wikipedia, The Free Encyclopedia. Accessed January 14, 2015. http://en.wikipedia.org/w/index.php?title=Accuracy_and_precision&oldid=641854421

Wilhelm, E., Battino, R., Wilcock, R.J. (1977) Low-pressure solubility of gases in liquid water. *Chemical Reviews*, 52, 219-262.

Xu, L., Furtaw, M.D., Madsen, R.A., Garcia, R.L., Anderson, D.J., McDermitt, D.K. (2006) On maintaining pressure equilibrium between a soil CO_2 flux chamber and the ambient air. *Journal of Geophysical Research-Atmospheres*, 111, D8.

Yagi, K., Tsuruta, H., Kanda, K., Minami, K. (1996) Effect of water management on methane emission from a Japanese rice paddy field: automated methane monitoring. *Global Biogeochem Cycles*, 10, 255-267.

Yano, M., Toyoda, S., Tokida, T., Hayashi, K., Hasegawa, T., Makabe, A., Koba, K., Yoshida, N. (2014) Isotopomer analysis of production, consumption and soil-to-atmosphere emission processes of N_2O at the beginning of paddy field irrigation. *Soil Biology and Biochemistry*, 70, 66-78.

Yao, H., Conrad, R., Wassmann, R., Neue, H.U. (1999) Effect of soil characteristics on sequential reduction and methane production in sixteen rice paddy soils from China, the Philippines, and Italy. *Biogeochemistry*, 47, 269-295.

Yuan, Q., Pump, J., Conrad, R. (2012) Partitioning of CH_4 and CO_2 production originating from rice straw, soil and root organic carbon in rice microcosms. *PLoS ONE*, 7, e49073.

Authors' affiliations

Lead authors

Kazunori Minamikawa
National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki, Japan
minakazu@affrc.go.jp

Takeshi Tokida
National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki, Japan
tokida@affrc.go.jp

Shigeto Sudo
National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki, Japan
ssudo@affrc.go.jp

Agnes Padre
International Rice Research Institute, Los Baños, Laguna, Philippines
a.padre@irri.org

Kazuyuki Yagi
National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki, Japan
kyagi@affrc.go.jp

Contributing authors

Prihasto Setyanto
Indonesian Agricultural Environment Research Institute, Pati, Central Java, Indonesia
prihasto_setyanto@yahoo.com

Tran Dang Hoa
Hue University of Agriculture and Forestry, Hue, Vietnam
trandanghoa@huaf.edu.vn

Amnat Chidthaisong
The Joint Graduate School of Energy & Environment, King Mongkut's University of Technology Thonburi, Bangkok, Thailand
amnat_c@jgsee.kmutt.ac.th

Evangeline B. Sibayan
Philippine Rice Research Institute, Muñoz, Nueva Ecija, Philippines
eb.sibayan@philrice.gov.ph

Yusuke Takata
National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki, Japan
takatay@affrc.go.jp

Takayoshi Yamaguchi
National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki, Japan
ladakh2008@affrc.go.jp

Reviewers

Kazuyuki Inubushi
Graduate School of Horticulture, Chiba University, Matsudo, Chiba, Japan
inubushi@faculty.chiba-u.jp

Reiner Wassmann
International Rice Research Institute, Los Baños, Laguna, Philippines
R.Wassmann@irri.org

Tetsuhisa Miwa
National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki, Japan
miwa@niaes.affrc.go.jp

Ngonidzashe Chirinda
International Center for Tropical Agriculture, Cali-Palmira, Colombia
n.chirinda@cgiar.org