GRIFFITH UNIVERSITY

Optimum sampling time and frequency for measuring N2O emissions from a rain-fed cereal cropping system

Author

Reeves, S, Wang, W

Published

2015

Journal Title

Science of the Total Environment

Version

Accepted Manuscript (AM)

DOI

10.1016/j.scitotenv.2015.05.117

Rights statement

© 2015, Elsevier. Licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International, which permits unrestricted, non-commercial use, distribution and reproduction in any medium, providing that the work is properly cited.

Downloaded from

http://hdl.handle.net/10072/102484

Griffith Research Online

https://research-repository.griffith.edu.au

Optimum Sampling Time and Frequency for Measuring N_2O Emissions from a Rain-fed Cereal Cropping System

STEVEN REEVES[1]* and WEIJIN WANG[1,2]
1. Department of Science, Information Technology and Innovation, Dutton Park, Qld, 4102, Australia. Tel: +61 7 3170 5710
2. Environmental Futures Research Institute, Griffith University, Nathan, Qld, 4111, Australia.
*Corresponding author at: 41 Boggo Road, Dutton Park, Qld, 4102, Australia. Tel: +61 7 3170 5710.
Email addresses: steven.reeves@qld.gov.au (S.Reeves), weijin.wang@qld.gov.au (W.Wang)
Keywords: nitrous oxide, diurnal variation, sampling frequency, static chamber

Abstract

Annual cumulative nitrous oxide (N_2O) emissions from soil have historically been calculated from intermittent data measured manually via the static chamber method. The temporal variability in emissions, both diurnally and between days, introduce uncertainty into the up-scaling of static chamber data. This study assessed the most appropriate time of day to sample and the best sampling frequency to ensure reliable estimates of annual cumulative emissions. Sub-daily N_2O emissions were measured using automatic gas sampling chambers over three years in a sub-tropical cereal crop system. The sub-daily dataset was divided into eight time periods per day to assess the best sampling time of day. Daily mean N_2O emissions were subsampled from the dataset to simulate different sampling frequencies, including pre-set and rainfall-based scenarios. Annual cumulative N_2O emissions were calculated for these scenarios and compared to the 'actual' annual cumulative emissions. The results demonstrated that manual sampling between mid-morning (09:00) and midday (12:00), and late evening (21:00) and midnight (24:00) best approximated the daily mean N_2O emission. Factoring in the need to sample during daylight hours, gas sampling from mid-morning to midday was the most appropriate sampling time. Overall, triweekly sampling provided the most accurate estimate (±4% error) of annual cumulative N_2O emissions, but was undesirable due to its labour intensive high sampling frequency. Weekly sampling with triweekly sampling in the two weeks following rainfall events was the most efficient sampling schedule, as it had similar accuracy (±5% error) to the triweekly sampling, the smallest variability in outcomes and approximately half the sampling times of triweekly sampling. Inter-annual rainfall variability affected the accuracy and variability of estimations of annual cumulative emissions, but did not affect the overall trends in sampling frequency accuracy. This study demonstrated that intermittent samplings are capable of estimating the annual cumulative N_2O emissions satisfactorily when timed appropriately.

1. Introduction

Nitrous Oxide (N_2O) is a potent greenhouse gas with a global warming potential 298 times that of carbon dioxide (IPCC, 2013). N_2O is also the largest ozone depleter in the atmosphere (Ravishankara et al., 2009). The concentration of N_2O in the atmosphere had increased by 20% in 2011 compared to 1750 levels (IPCC, 2013). This increase has primarily been attributed to agricultural sources, dominated by emissions from soil supplied with synthetic and/or organic fertilisers (IPCC, 2013; Syakila and Kroeze, 2011; Davidson, 2009). Consequently there has been a concerted effort to quantify annual emissions from soil in different agro-ecosystems.

There are a number of methods for measuring N_2O emissions from soil (Dalal et al., 2003). Historically, the static chamber method has been employed most widely. The static chamber method involves inserting a base into the soil, and placing a closure over the top for a set period of time. Gas samples are manually extracted from the headspace using a syringe and injected into sealed containers for laboratory analysis. Compared to other methods (e.g. micrometeorological and automatic sampling systems), static chambers have a lower initial setup cost, are simple to

deploy in the field, easily lend themselves to increased replication and can be used in smaller experimental plots often favoured in agricultural experiments.

One of the main disadvantages of the static chamber method is its labour intensiveness. In most cases, this limits sampling to once per day during daylight hours, which could overlook diurnal variation in emissions. Diurnal variation in N_2O emissions have been observed in a number of environments (Williams et al., 1999; Flessa et al., 2002; Du et al., 2006; Alves et al., 2012; Morris et al., 2013). Consequently, measuring only once per day, could produce an over- or underestimation in calculated daily emissions, depending on when the measurement is taken.

As well as diurnal variation, day to day N_2O emission variability of several orders of magnitude have been recorded in many environments (Allen et al., 2010, Wang et al., 2011, Yao et al., 2009) and presents a challenge to utilising static chamber data to produce annual cumulative emission estimates. Manually sampling on a daily basis to obtain a yearlong cumulative emission measurement is cost prohibitive and impractical. To address this, many different pre-set sampling regimes have been used, ranging from fortnightly sampling (e.g. Williams et al., 1999) to 3 times per month (e.g. Mu et al., 2013), with interpolation between sampling dates to calculate annual cumulative emissions. However, it has been widely reported that in many agricultural systems, N_2O emissions are episodic and linked to events such as rainfall, irrigation or fertiliser application, which may be missed by a pre-set sampling regime. If sampling falls on a high emission day, interpolation may overestimate emissions. Conversely, if sampling falls on a low emission day, the cumulative emissions may be underestimated. With this in mind, some studies have sought to account for these event-based emissions by increasing sampling during expected high emission periods and reducing sampling during expected low emission periods (Wang et al., 2011; Luo et al., 2013; De Klein et al., 2003; Zhang et al., 2014; Smith et al., 2012). However, the sampling time and frequency in the event-based measurements are often determined arbitrarily by the operators and thus are short of scrutiny based on scientific data.

To eliminate bias associated with temporal variability, continuous automatic chamber systems have been developed and their use has increased within the last decade (Smith et al., 2001; Livesley et al., 2011; Scheer et al., 2012; Barton et al., 2013; Fassbinder et al., 2013; Kennedy et al., 2013; Van Der Weerden et al., 2013). However, the initial setup and ongoing costs involved with running an automatic system, the limited number of chambers available, the requirements for adequate monitoring and maintenance, and the risk of prolonged data loss in the event of instrument failure mean that the static manual chamber method would still be used extensively in the field. With this in mind, it is important to assess appropriate sampling frequencies and inform future sampling regimes so that temporal variability is accounted for, and the collected emission data can confidently be used to calculate annual cumulative N_2O emissions. Emission data from continuous automatic chamber systems presents a unique opportunity to evaluate diurnal and day to day variability in N_2O emissions, and thus inform static chamber sampling regimes.

In this paper we use sub-daily automatic chamber emission data collected over three years from a subtropical rain fed wheat cropping site to (i) assess what time of day most accurately approximates the daily mean N_2O emission for this system and (ii) simulate a number of different gas sampling schemes, including pre-set and rainfall based, to determine the most efficient, practicable and reliable sampling frequency to measure the annual cumulative N_2O emission for this system.

2. Materials and Methods

2.1. Experimental site and treatments

The experimental site is located at the Hermitage Research Station (28°12'S, 152°06'E), southern Queensland, Australia. A long-term field experiment to investigate the effects of different management practices on soil properties and wheat/barley crop yields has been in place since December 1968. There were twelve treatments in factorial combination of conventional tillage (CT) *vs*. no-till (NT), stubble retention (SR) vs. stubble burning (SB), and three N fertiliser (urea) application rates at 0 (0N), 60 kg N ha^{-1} yr^{-1} (60N) and 90 kg N ha^{-1} yr^{-1} (90N). Long-term (1970-2000) mean annual temperature at the site was 17.2°C, with the lowest mean monthly temperature (10.1°C) in July, and the highest (23.2°C) in January. Annual precipitation over the study period was 489 mm, 857 mm and 713 mm for 2006-2007, 2007-2008 and 2008-2009, respectively. Long-term mean annual rainfall was 728 mm, about 60% of which is received between October and February. The soil is a Vertosol (Isbell, 2002) with 65% clay, 24% silt, 11% sand, ~20.0 mg g^{-1} organic carbon and a bulk density of 1.05 g cm^{-3} on average in the 0-20 cm layer. More detailed site description and the original experimental design were given by Wang et al (2011).

2.2. Nitrous Oxide Measurements

Measurements of N_2O emissions began on the 1/07/2006 and finished on the 30/06/2009, encompassing three wheat-fallow cycles. There were a total of 9 chambers in the automatic sampling system with three chambers deployed in the CT-SR-90N treatment and two in the NT-SR-90N treatment (Wang et al., 2011). The remaining 4 chambers were deployed in treatments with no N fertiliser addition, and were not used for this analysis because N_2O emissions under 0N were generally low. Each chamber consisted of a 500 x 500 x 150 mm stainless steel base inserted into the soil, a 500 x 500 x 300 mm perspex open ended extension, and a 500 x 500 x 300 mm cover box with two opening panels. Each chamber covered two rows of wheat and the extension was only in place when the wheat plant exceeded the height of the cover box. Chambers were closed in a set of three, and headspace gas extracted from each chamber consecutively at 3 minute intervals for 48 minutes. During that time four samples were extracted from each chamber to calculate an emission flux. After every three head space samples, a calibration standard was analysed (N_2O: 0.5 ppm, CH_4: 2 ppm and CO_2 500 ppm; BOC Ltd, Sydney, Australia). All samples were analysed on site with a gas chromatograph (SRI 8610C, SRI Instruments, CA, Torrance, USA) fitted with an Electron Capture Detector (ECD). Samples passed through an ascarite pre-column to remove carbon dioxide before being separated on a packed column (Haye Sep N; Mesh 60/80; diameter 1/8"; length 6 ft) using N_2 as the carrier gas. Column oven and ECD temperatures were 50°C and 330°C, respectively.

Air temperature and rainfall were recorded at a Bureau of Meteorology site located within 1 km of the field site. Soil temperature at a depth of 5-7cm was logged at 15 minute intervals using temperature probes (Measurement Engineering Australia, SA, Australia).

2.3. Data Analysis

N_2O emissions were calculated from the increase of gas concentration in the headspace using linear regression (Wang et al., 2011). Daily means were calculated from 10 sub-daily N_2O emissions measured at 144-minute intervals over a 24 hr period. Some daily means were calculated from fewer emission values as those measurements with R^2<0.80 in the linear regression were removed. Annual cumulative emissions were calculated as the sum of all daily means. Emission values for days that had no emission values due to instrument breakdown were interpolated from days on both sides. However, in instances where emission data was missing on days leading to a rainfall event, the earlier lower daily emission value was extended to the start of the rainfall event, to avoid any overestimation of emissions caused by interpolation with a high emitting rainfall event. Annual cumulative emission values were calculated for the three crop-fallow cycles 01/07/2006 to 30/06/2007, 01/07/2007 to 30/06/2008 and 01/07/2008 to 30/06/2009. These cumulative values were considered the 'actual' cumulative N_2O emissions for assessing other less frequent sampling schedules.

To assess diurnal variability in N_2O emissions, the daily 24 hr time period was divided into 8 equal measurement periods of 3 hours each. Individual emission rates were then allocated to a 3 hr time period based on when the data was measured. Only the CT-SR-90N treatment was used for the diurnal analysis as it had the largest range of emissions and three replicates as compared to two for other treatments (Wang et al., 2011). Emissions data lower than the detection limit of the system (~2 g N_2O-N ha^{-1} d^{-1}) were not included, with the remaining categorised as either low emission (2-20 g N_2O-N ha^{-1} d^{-1}) or high emission (>20 g N_2O-N ha^{-1} d^{-1}) days. Those days that had ≥3 missing measurements were excluded in the analysis to avoid biasing diurnal data for that day. In cases where 1-2 measurements were missed in a day, the missing measurements were estimated by interpolation before the diurnal analysis. To determine which sampling time period best approximated the daily mean N_2O emission rate (E_d), the N_2O emissions measured in each time period (E_t) was assessed as follows:

$$D_t = \left(\frac{E_t - E_d}{E_d}\right) * 100 \quad (1)$$

D_t is the difference between E_t and E_d as a percentage of E_d and indicates whether E_t over- or underestimated E_d.

The complete set of daily mean N_2O emissions were sub-sampled to simulate a number of pre-set and event-based gas sampling schedules (Table 1). Only rainfall events of >20 mm per day were considered the trigger in the event-based schedules because lower rainfall events and N fertiliser application alone did not significantly enhance N_2O emission in this cropping system (Wang et al., 2011). To best approximate realistic manual sampling activities, Saturdays and Sundays were excluded. However, when rainfall events of >20 mm occurred on a Friday or a Saturday, the following day was sampled in the event-based sampling schedules. From the sub-sampled data in each sampling schedule, annual cumulative emissions were calculated over the same time periods as used to calculate the 'actual' annual cumulative N_2O emissions. For the event-based sampling schedules, the sampled emission rate prior to >20 mm rainfall was extended up to the start of the rainfall event when annual cumulative emissions were calculated. Simple interpolation was used for the pre-set sampling schedules, as is normal practice. Each sampling schedule had a number of possible outcomes depending on which day of a week gas sampling was undertaken (Table 1).

Table. 1. Simulated sampling schedules

Sampling Schedule	Frequency	Samplings per year	Outcomes Chamber^{-1} Year^{-1}
Triweekly	Monday, Wednesday & Friday.	156	1
Biweekly	Monday & Thursday; or Tuesday & Friday.	104	2
Weekly	Monday; or Tuesday; or Wednesday; or Thursday; or Friday.	52	5
Fortnightly	Fortnightly cycle of Monday; or Tuesday; or Wednesday; or Thursday; or Friday.	26	10
Weekly + Triweekly	As per weekly sampling, but triweekly for 2 weeks after >20 mm daily rainfall.	69-86*	5
Weekly + Biweekly	As per weekly sampling, but biweekly for 2 weeks after >20 mm daily rainfall.	59-77*	10
Fortnightly + Triweekly	As per fortnightly sampling, but triweekly for 2 weeks after >20 mm daily rainfall.	48-70*	10

Fortnightly + Biweekly	As per fortnightly sampling, but biweekly for 2 weeks after >20 mm daily rainfall.	38-53*	20

*Range of samplings per year over 3 years, depending on number of > 20 mm rainfall events in a year.

The performance of different simulated manual gas sampling schedules was assessed using the 'actual' annual cumulative N_2O emissions as the benchmark. The deviation (D_s) of the simulated (E_s) from the 'actual' (E_a) annual cumulative N_2O emission was calculated for each sampling chamber by:

$$D_s = \left(\frac{|E_s - E_a|}{E_a}\right) * 100 \quad (2)$$

where $|E_s - E_a|$ is the absolute difference between the simulated and the 'actual' annual cumulative emissions. D_s indicates, as a percentage of E_a, how close E_s estimated E_a for a single sampling schedule outcome. D_s was found to follow a non-normal distribution, as shown by the Shapiro-Wilk test (W =0 .5811, $P < 0.001$). To compare different sampling schedule outcomes, the non-parametric Kruskal-Wallis ANOVA was used on the non-transformed data, followed by Dunn's post hoc pairwise multiple comparison procedure with Bonferroni correction. Statistical Analysis was undertaken using R (R Core Team, 2014) and R Package Dunn.test (Dinno, 2015).

The probability, P_a of a sampling schedule having an outcome within a given precision of the 'actual' annual cumulative emission was calculated by:

$$P_a = \left(\frac{N_a}{N}\right) * 100 \quad (3)$$

where the total number of outcomes within a defined precision (N_a), is given as a percentage of the total number of outcomes possible for that sampling schedule (N).

3. Results

3.1. Daily Dynamics of N_2O Emissions

Over the three-year study period, N_2O emissions exhibited significant daily, seasonal and yearly variation (Fig. 1). Large peaks of N_2O emissions (>20 g N_2O-N ha^{-1} d^{-1}) that lasted up to two weeks, generally occurred following large (>20 mm) rainfall events. As these rainfall events generally occurred during spring and summer, the majority of large emissions occurred in this time period. Based on the complete sub-daily datasets, the CT-SR-90N treatment had annual cumulative emissions (mean ± SE) of 716 ± 48 g N_2O-N ha^{-1} yr^{-1}, 1267 ± 201 g N_2O-N ha^{-1} yr^{-1}, and 1941 ± 251 g N_2O-N ha^{-1} yr^{-1} for 2006-2007, 2007-2008 and 2008-2009 respectively. The NT-SR-90N had annual cumulative emissions (mean ± SE) of 506 ± 10 g N_2O-N ha^{-1} yr^{-1}, 1683 ± 1320 g N_2O-N ha^{-1} yr^{-1} and 652 ± 267 g N_2O-N ha^{-1} yr^{-1} for 2006-2007, 2007-2008 and 2008-2009 respectively (Wang et al., 2011).

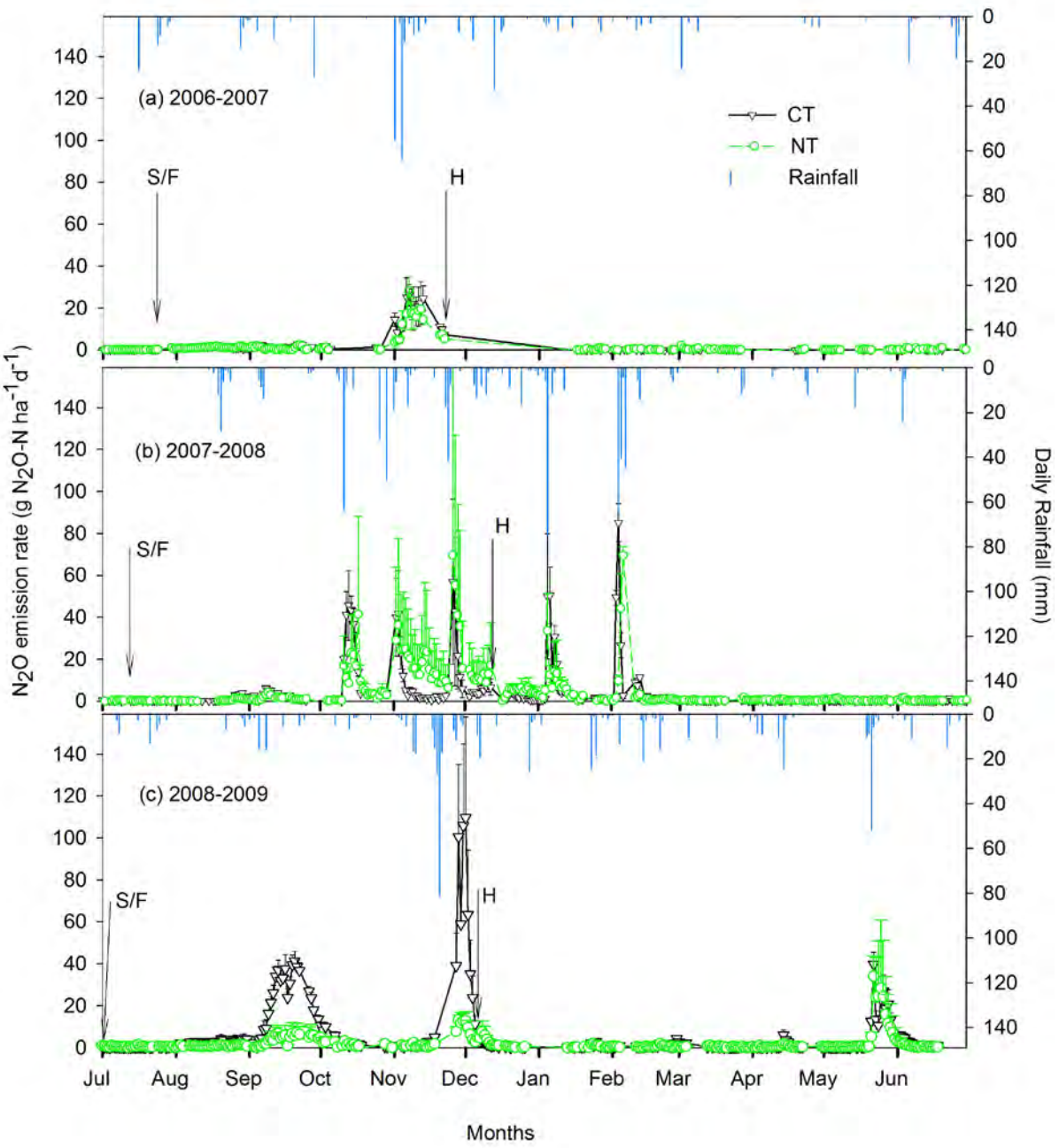

Fig. 1. Daily N_2O emissions (mean ± SE) and rainfall over (a) 2006-2007, (b) 2007-2008 and (c) 2008-2009 years from conventional till (CT) and zero till (NT) treatments applied with 90 kg N ha^{-1} yr^{-1} as urea. Arrows show sowing/fertilizer application (S/F) and harvest (H) date.

3.2. Diurnal Variability in N_2O Emissions

Diurnal variability in N_2O emissions was generally evident in the data during high (>20 g N_2O-N ha^{-1} d^{-1}) emitting periods (Fig. 2). The diurnal fluctuation did not always follow the same pattern on different days (data not shown). However, averaged across all the high emission days, diurnal variation in N_2O emissions from the daily mean demonstrated a pronounced sinusoidal pattern (Fig. 2a). Highest overestimation of approximately 20% of the daily mean N_2O emissions was recorded from mid to late afternoon (15:00 to 18:00) and the largest underestimation of approximately 15% was recorded from late night to early morning (03:00 to 09:00). Measurements from 09:00 to 12:00

and from 21:00 to 24:00 most closely approximated the daily mean N_2O emission on high emission days. In contrast to the clear trend associated with high emission days, low emission days did not exhibit clear evidence of diurnal cycling, and trends across individual replicates were less consistent (Fig. 2b).

The diurnal variation in N_2O emissions on high emission days closely followed the daily cycle of air and soil temperature (Fig. 2c). Maximum air temperature occurred mid-afternoon, with maximum soil temperature lagging ~1 hour behind at 15:00, coinciding with the largest overestimation of emissions from 15:00 to 18:00. Similarly, minimum air and soil temperature approximately coincided with the largest underestimation of N_2O emissions. Mean daily soil temperature (19.2°C) occurred within the two periods (09:00-12:00 and 21:00- 24:00) identified as most closely estimating the daily mean N_2O emissions, while the daily mean air temperature occurred 1-2 hours prior to the optimal sampling time periods.

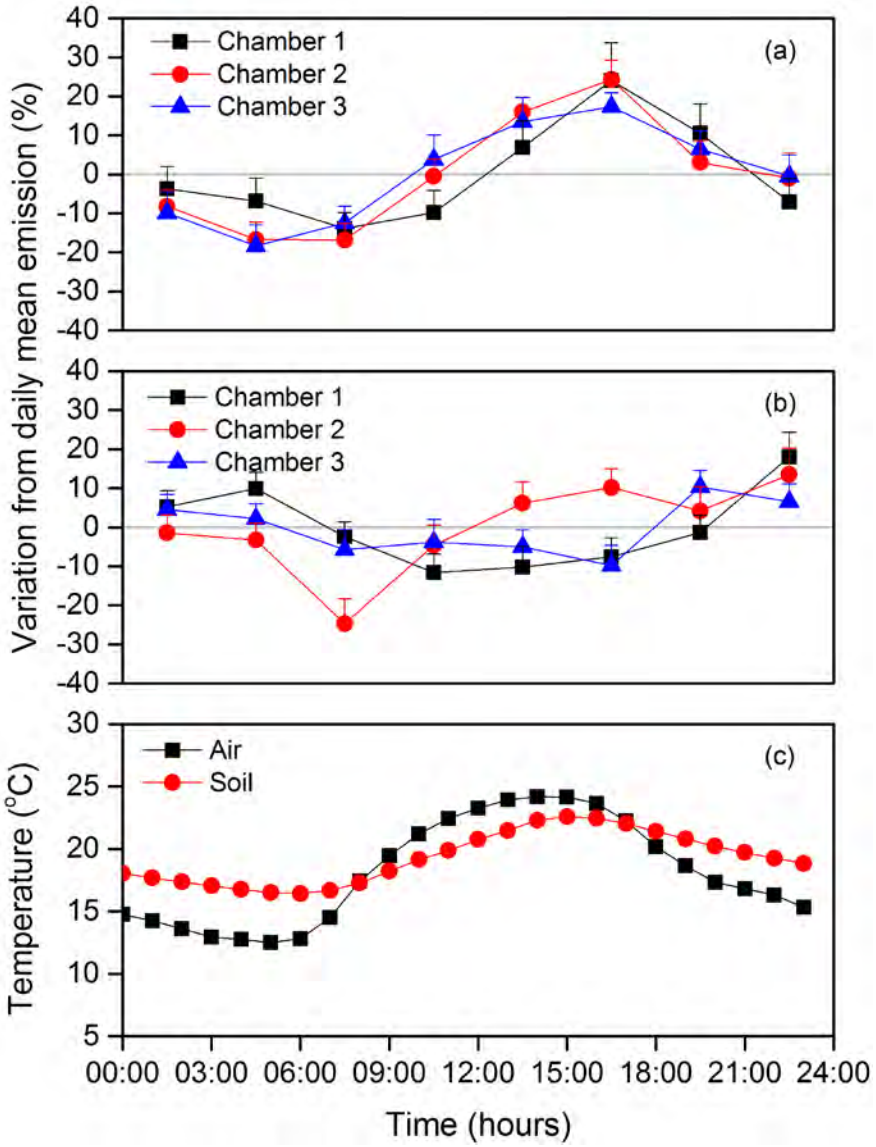

Fig. 2. Diurnal variation (D_t) in N_2O emission rates during high emission days (a: >20 g N_2O-N ha^{-1} d^{-1}) and low emission days (b: 2-20 g N_2O-N ha^{-1} d^{-1}). The vertical bars are standard error of means for a sampling time period across different days. Diurnal variation in air and soil temperature (at 5cm depth) for high emission days (c).

A regression analysis confirmed a close 1:1 relationship between daily N_2O emissions estimated using 09:00-12:00 measurements and their corresponding "actual" daily mean N_2O emissions (Fig.

3). Taking into account operational convenience, 09:00-12:00 would be considered as the optimum gas sampling time.

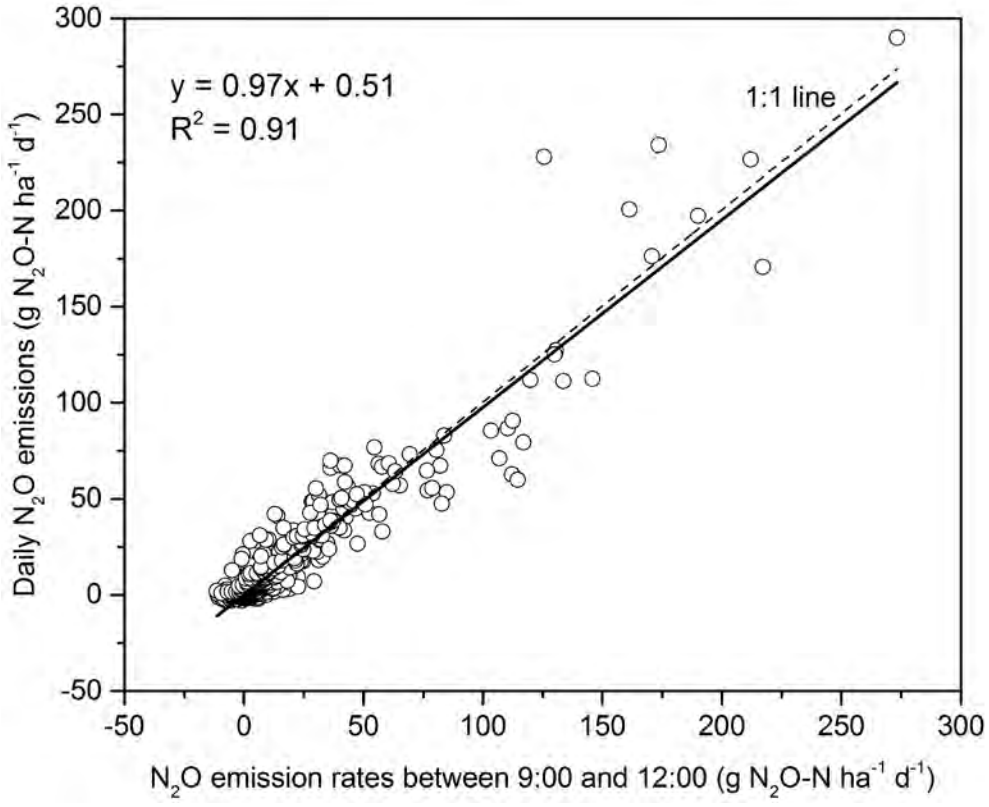

Fig. 3. Relationship between "actual" daily mean N_2O emissions and the N_2O emissions estimated using measurements during the period from 09:00 to 12:00.

3.3. Impacts of Sampling Frequencies on Annual Cumulative N_2O Emissions

There was no consistent trend of over- or underestimation of the 'actual' annual cumulative N_2O emissions for a specific intermittent sampling schedule (Fig. 4). The potential outcomes were generally spread around the 'actual' annual cumulative N_2O emission value.

The variation (stdev) in possible outcomes was markedly different across sampling schedules (Fig. 4). Consistently, the variation in potential outcomes was largest for Fortnightly samplings, with variations averaged across years of 430 g N_2O-N ha^{-1} yr^{-1}. While the smallest variation, averaged across years, occurred in the Weekly + Triweekly sampling schedule with 33 g N_2O-N ha^{-1} yr^{-1}. Largest variations across all sampling schedules occurred in 2007-2008 (28 – 807 g N_2O-N ha^{-1} yr^{-1}), while the smallest were in 2006-2007 (15 – 80 g N_2O-N ha^{-1} yr^{-1}).

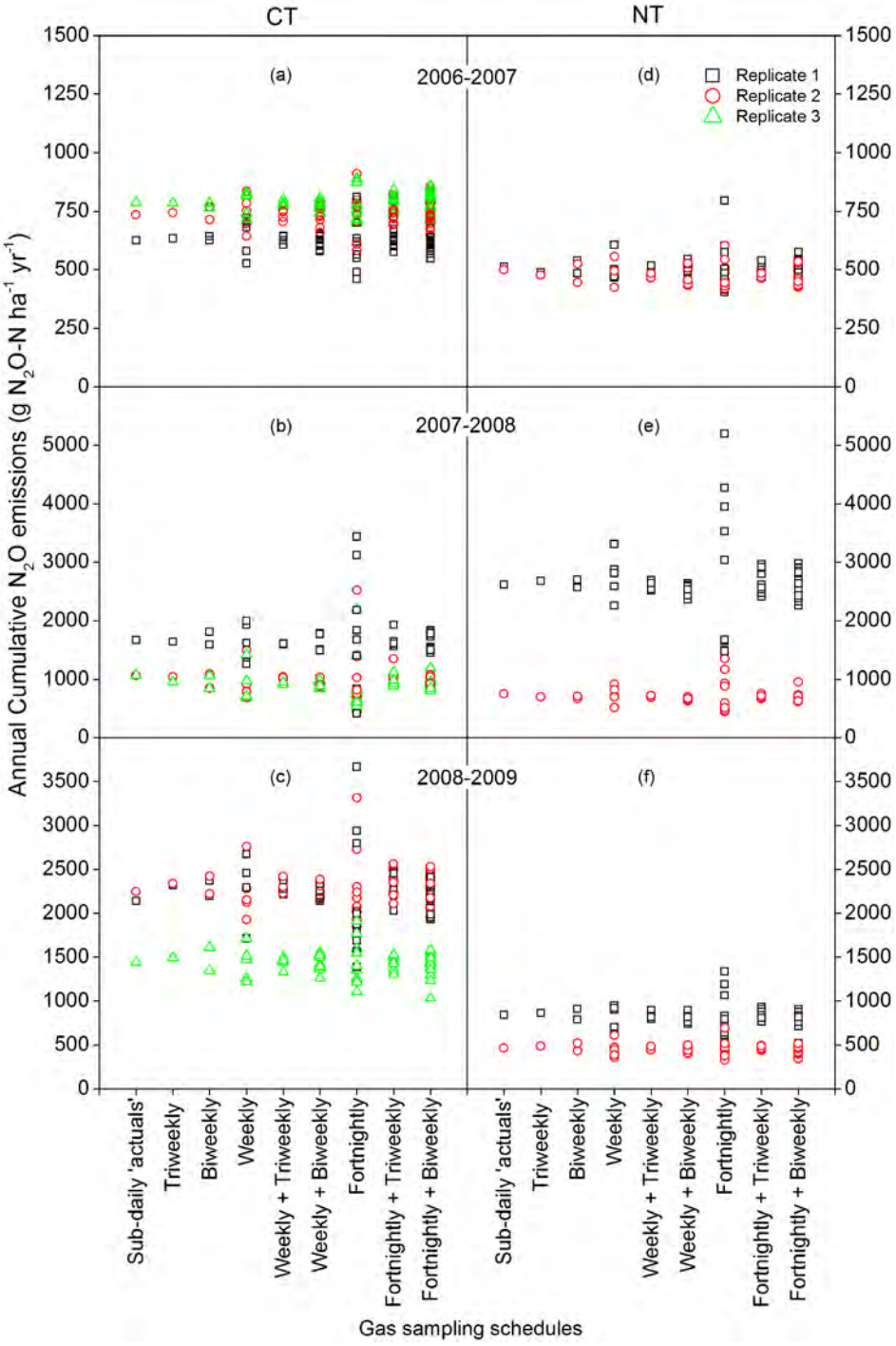

Fig. 4. Scatter plots showing the possible annual cumulative N_2O emissions from individual chambers (3 chambers for CT and 2 chamber for NT) of all sampling schedules for treatments CT-SR-90N (a, b and c) and NT-SR-90N (d, e and f) in the 2006-2007, 2007-2008 and 2008-2009 years.

Kruskal-Wallis ANOVAs showed significant differences between ranked estimations obtained from sampling schedules for all individual years (2006-2007: $P < 0.001$; 2007/2008: $P < 0.001$; 2008/2009: $P < 0.001$) and over three years ($P < 0.001$). The accuracy of estimation of annual cumulative N_2O emissions was generally improved with increasing number of samplings per year (Fig. 5). Over the three years, outcomes from the Weekly and Fortnightly sampling schedules were significantly poorer ($P < 0.05$) than all other sampling schedules. Increasing the sampling frequency to biweekly or triweekly after rainfall substantially reduced the errors in the estimated annual cumulative N_2O emissions. For example, increasing sampling in a Weekly schedule to two or three times per week following >20 mm daily rainfall increased the estimation accuracy over the three years by 8% and 9%, respectively. Estimations, over the three years, based on fortnightly samplings were improved

by 18% and 20%, when biweekly and triweekly samplings, respectively, were applied after daily rainfall of >20 mm. Of the rainfall-based sampling schedules, Weekly + Triweekly and Fortnightly + Triweekly consistently delivered estimates with errors <±7% in all years, while the Weekly + Biweekly and Fortnightly + Biweekly schedules appeared less satisfactory in 2007-2008 with the errors reaching ±10%.

Over the three years and within individual years, the errors in estimated annual N_2O emissions from the Weekly + Triweekly and Fortnightly + Triweekly sampling schedules and the pre-set Biweekly and Triweekly schedules were not statistically different from one another. With approximately 18-56 more sampling times per year than the Weekly + Triweekly and Fortnightly + Triweekly schedules respectively, the Biweekly sampling schedule did not result in more accurate annual N_2O emissions in any year. The Triweekly sampling schedule consistently delivered the most accurate estimates. However, the outcomes from this most intensive sampling schedule (156 times per year) were not significantly better than those obtained with the Weekly + Triweekly and Fortnightly + Triweekly schedules.

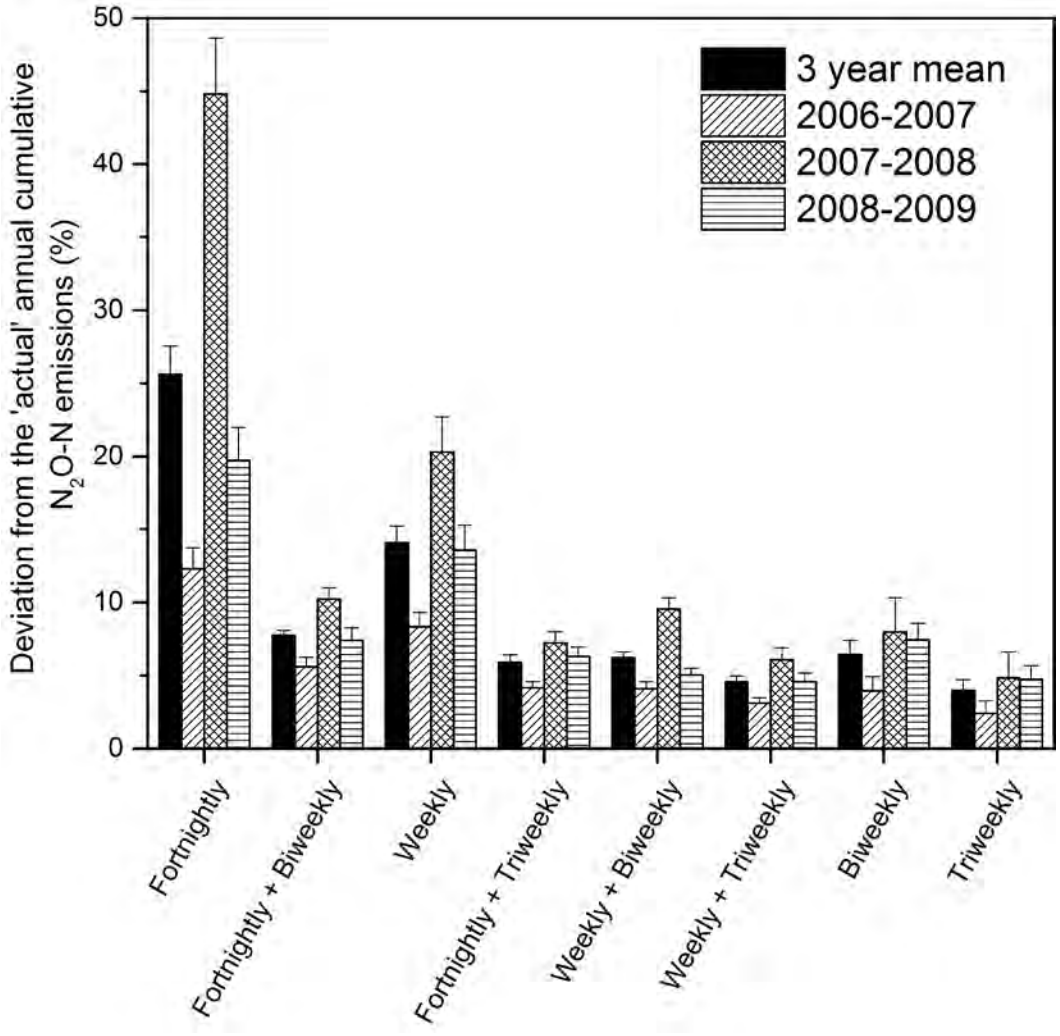

Fig. 5. Mean percent deviation from the 'actual' annual cumulative N_2O emissions for different sampling schedules. Data are combined means from CT-SR-90N and NT-SR-90N treatments across three years and for individual years. Sampling schedules are arranged in increasing sampling frequency. Vertical lines are standard error of multiple outcomes.

Over the 3 years, the probability of estimating the 'actual' annual cumulative N_2O emissions within 10% precision was highest for Triweekly (93%) and Weekly + Triweekly (91%) samplings (Fig. 6a). These two sampling schedules also had the highest probabilities at 10% precision in 2006-2007

(100%) and 2007-2008 (80%), while the Weekly + Triweekly and Weekly + Biweekly had the highest probabilities in 2008-2009 (92%) (Fig. 6b,c,d). Weekly and Fortnightly sampling schedules consistently had the lowest probabilities in each year. Generally, a sampling schedule at a given precision had a higher probability of estimating the 'actual' annual cumulative N_2O emissions in 2006-2007, compared to 2007-2008 and 2008-2009.

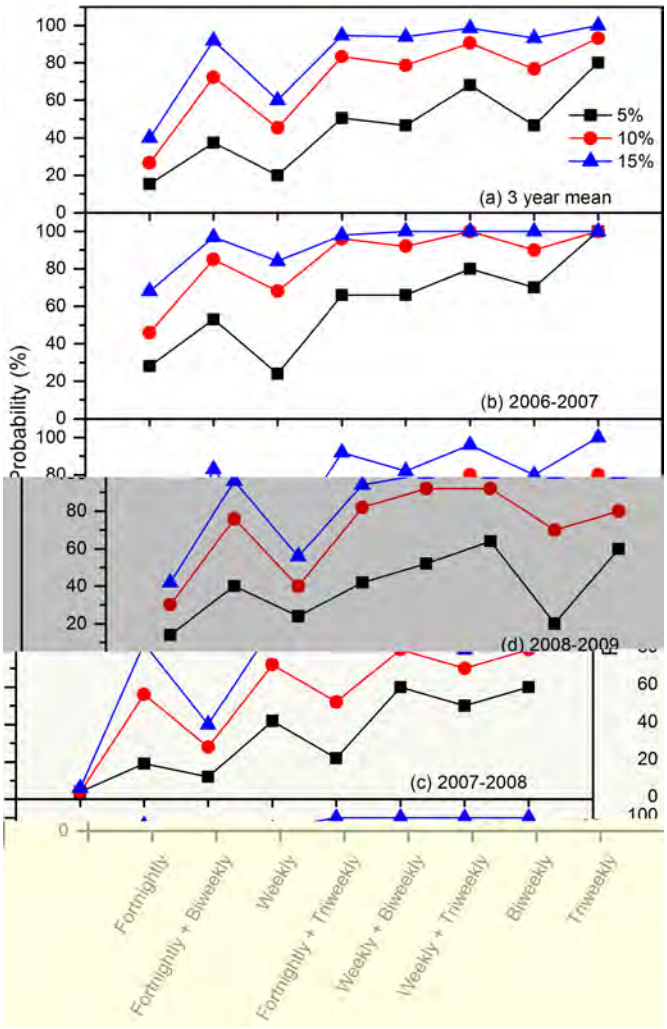

Fig. 6. The probabilities of obtaining an annual cumulative emission value within a given precision of the 'actual' annual cumulative emission, for different sampling schedules for individual years and over three years. CT-SR-90N and NT-SR-90N data are combined. Sampling schedules are arranged in increasing sampling frequency from left to right.

4. Discussion

4.1. Diurnal variation in N_2O emissions and implication for best gas sampling times of day

Diurnal variability in N_2O emissions has been reported in many studies (Denmead et al., 1979; Blackmer et al., 1982; Akiyama et al., 2000; Maljanen et al., 2002; Alves et al., 2012; Van Der Weerden et al., 2013) and is supported by our observations on days with high emissions (>20 g N_2O-N ha^{-1} d^{-1}). There was no clear evidence of diurnal cycling on days with low emissions in our study, indicating that either there were no diurnal fluctuations or those fluctuations were insignificant. These observed differences are because N_2O production, through nitrification and/or denitrification, is interactively regulated by many soil and environmental factors, such as substrate (NH_4^+ and NO_3^-), temperature, water content and oxygen supply (Dalal et al., 2003), and the limiting factor can

change through time. Soil temperature has consistently been reported as the main underlying factor responsible for diurnal cycles in N_2O emissions, however it does not always exert influence on N_2O emissions (Denmead et al., 1979; Maljanen et al., 2002; Van Der Weerden et. al., 2013). In the case of high emission days, factors such as abundant substrate and moderate soil water content provide optimum conditions for N_2O production, thus allowing temperature to become the predominant limiting factor. However, on low emission days inadequate substrate and/or low soil water content may constrain N_2O production, making emissions less sensitive to diurnal changes in temperature.

The exact temperature mechanism that causes the diurnal emissions response, in instances when other soil and environmental factors are non-limiting, is generally considered to be changes in microbial activity (denitrification and oxygen consumption) with temperature. These changes include both an increase in the rate of denitrification per unit of anaerobic volume and the expansion of anaerobic zones, through increased respiration, which reduces soil oxygen content (Smith et al., 2003). However, Blackmer et al. (1982) suggested that microbial activity alone could not explain the large amplitudes in daily cycles of N_2O emission, instead theorising that it could also be temperature-induced changes in the solubility of N_2O in soil water. These hypotheses could explain the diurnal cycles in N_2O emissions in our study on high emission days as they were recorded mainly after rainfall when soil water content had increased (ranging from 47 to 90% WFPS, data not shown).

Even though the presence of a diurnal cycle in N_2O emissions is not consistent through time, its presence on any particular day means manual gas sampling has the potential to bias cumulative N_2O emission measurements if sampled at the wrong time of day. This is especially true given that the high emission days generally account for large proportions in annual emissions. At our site, the time periods that best estimated the daily mean N_2O emission rate were from mid-morning to midday and late evening to midnight (Fig. 2), with mid-morning to midday considered the best sampling time period due to constraints with conducting fieldwork in the dark. Other studies have also concluded that morning time periods, including 09:00 to 10:00 (Alves et al., 2012, Savage et al., 2014) and 10:00 to 12:00 (Van Der Weerden et al., 2013) are the best time to manually sample static chambers. However, some studies have found large underestimations for samplings during 09:00 to 11:00 (Scheer et al., 2012) and overestimations during 09:00 to 12:00 (Scheer et al., 2014).

4.2. Daily variation in N_2O emissions and appropriate gas sampling frequency

Alongside selecting the most representative time period within a 24 hour period for manual gas sampling, it is vital to apply a sampling schedule which is capable of providing a good estimate of the annual cumulative N_2O emission. Other studies have used data collected from several months to a year, to examine only pre-set manual sampling schedules from 1 – 31 days (Parkin, 2008; Liu et al., 2010; Morris et al., 2013). An exception has been Rowlings et al (2015) who assessed pre-set sampling intervals and one rainfall based sampling schedule over two years. The current study is perhaps the longest sub-daily N_2O emission data set that has been used to evaluate the effectiveness of both pre-set and rainfall based manual gas sampling frequencies. It therefore encompassed a large variety of emission periods, providing a sound representation of seasonal and inter-annual variability.

The accuracy of individual sampling schedules was mainly influenced by the ability of a schedule to representatively sample both high and low emission periods. Therefore, increasing sampling frequency generally increased the accuracy of annual cumulative emission estimations. Parkin (2008) reported similar findings for sampling intervals ranging from 1 to 22 days. Furthermore, increased sampling frequency minimised overestimations caused by interpolation of sampled emission peaks across days when no sample was taken. For this reason, the fortnightly and weekly sampling schedules, were significantly less accurate and more variable than other pre-set sampling schedules (i.e. Triweekly and Biweekly).

Similarly, all rainfall-based sampling schedules, due to their targeted increased sampling frequency during emission peaks, significantly increased the accuracy of estimations to levels similar to the most accurate sampling schedule (i.e. Triweekly sampling schedule). Therefore, increasing the frequency of sampling after rainfall events would be a recommended addition to pre-set sampling schedules. Van der Weerden et al. (2013) also recommended increasing samplings after rain events, although they used a 10 mm d^{-1} rainfall event as a trigger, compared to the 20 mm d^{-1} trigger in our study. In contrast, Rowlings et al. (2015) reported an increased potential error and an overestimation of emissions from fortnightly sampling with increased sampling after rainfall, compared to only fortnightly sampling. The reason for this difference could be caused by the interpolation method utilised between pre-rainfall low emission periods and post-rainfall high emission events. In the current study, low emission samplings before rainfall were extended up to the start of a rainfall period to avoid overestimation. Therefore the effectiveness of rainfall-based sampling schedules is greatly influenced by the interpolation method employed during data processing, and the rainfall trigger amount chosen, which could be different for different soil conditions.

Inter-annual rainfall variability affected the accuracy of sampling schedules. All sampling schedules exhibited the highest accuracy and smallest variability in the driest year, 2006-2007, and the lowest accuracy and highest variability in the wettest year, 2007-2008. It is likely that with an increase in rainfall events, and therefore an increase in the number of high emission periods, there is a larger possibility of not representatively sampling emissions. Even though the accuracy and variability of sampling schedule outcomes changed annually, the relative trends amongst sampling schedules remained similar.

It is important to strike a balance between sampling intensity and the accuracy required in the estimate of the annual cumulative N_2O emission. Though the Triweekly sampling was the most accurate overall, it was also the most demanding sampling schedule and thus undesirable due to its labour intensive nature. Assuming 10% precision as an acceptable level of error in annual cumulative emissions the Weekly + Triweekly sampling schedule was the only schedule to achieve the same or higher level of probability in each year as the Triweekly sampling schedule. Therefore, the Weekly + Triweekly sampling schedule was more efficient than triweekly sampling as it achieved a similar level of accuracy to triweekly sampling with half the sampling intensity. Furthermore, the Weekly + Triweekly sampling had the smallest variation in estimation outcomes, indicating the day of week chosen to be sampled during the weekly component did not greatly alter the outcome. Overall, the weekly with triweekly sampling after rainfall sampling schedule would be the most efficient sampling schedule.

5. Conclusion

This study has demonstrated that the annual cumulative N_2O emission can be satisfactorily estimated by intermittent samplings. Using long-term sub-daily N_2O emission data, this study has assessed the most appropriate time of day to sample N_2O emissions and the influence of sampling frequency on annual cumulative emission estimates in rain-fed subtropical winter crops. At this site, manual sampling between mid-morning and midday, and late evening to midnight best approximated the daily mean N_2O emission. However, considering the need to sample during daylight hours, gas sampling from mid-morning to midday was concluded as the most appropriate sampling time. Triweekly sampling was the most accurate sampling schedule to estimate the annual cumulative N_2O emissions, with ±4% error, although its high sampling intensity was undesirable. weekly sampling with triweekly sampling in the two weeks following rainfall events had similar accuracy (±5% error) to triweekly sampling over the three years, but with half the sampling intensity. Also, the probability of estimating the annual cumulative emissions with 10% precision was equal to or higher than that from triweekly sampling across all three individual years. Therefore weekly

sampling with triweekly sampling following rainfall events was the most efficient sampling schedule, and is recommended for future sampling campaigns.

Acknowledgments

This project was funded by the Department of Climate Change and Energy Efficiency (DCCEE). We are grateful to Grant Pu and Phil Bloesch for their constructive comments in reviewing an early version of this paper.

References

Akiyama H, Tsuruta H, Watanabe T. 2000. N2O and NO emissions from soils after the application of different chemical fertilizers. Chemosphere - Global Change Science 2(3-4):313-20.

Allen D, Kingston G, Rennenberg H, Dalal R, Schmidt S. 2010. Effect of nitrogen fertilizer management and waterlogging on nitrous oxide emission from subtropical sugarcane soils. Agriculture, Ecosystems and Environment 136:209-217.

Alves BJR, Smith KA, Flores RA, Cardoso AS, Oliveira WRD, Jantalia CP, Urquiaga S, Boddey RM. 2012. Selection of the most suitable sampling time for static chambers for the estimation of daily mean N 2O flux from soils. Soil Biology and Biochemistry 46:129-35.

Barton L, Murphy DV, Butterbach-Bahl K. 2013. Influence of crop rotation and liming on greenhouse gas emissions from a semi-arid soil. Agriculture, Ecosystems and Environment 167:23-32.

Blackmer AM, Robbins SG, Bremner JM. 1982. Diurnal variability in rate of emission of nitrous oxide from soils. Soil Science Society of America Journal 46(5):937-42.

Dalal RC, Wang W, Robertson GP, Parton WJ. 2003. Nitrous oxide emission from Australian agricultural lands and mitigation options: A review. Australian Journal of Soil Research 41(2):165-95.

Davidson EA. 2009. The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860. Nature Geoscience 2(9):659-62.

De Klein CAM, Barton L, Sherlock RR, Li Z, Littlejohn RP. 2003. Estimating a nitrous oxide emission factor for animal urine from some New Zealand pastoral soils. Australian Journal of Soil Research 41(3):381-99.

Denmead OT, Freney JR, Simpson JR. 1979. Studies of nitrous oxide emission from a grass sward. Soil Science Society of America Journal 43(4):726-8.

Dinno, A. 2015. dunn.test: Dunn's Test of Multiple Comparisons Using Rank Sums. R package version 1.2.2. URL http://CRAN.R-project.org/package=dunn.test.

Du R, Lu D, Wang G. 2006. Diurnal, seasonal, and inter-annual variations of N2O fluxes from native semi-arid grassland soils of inner Mongolia. Soil Biology and Biochemistry 38(12):3474-82.

Fassbinder JJ, Schultz NM, Baker JM, Griffis TJ. 2013. Automated, low-power chamber system for measuring nitrous oxide emissions. Journal of Environmental Quality 42(2):606-14.

Flessa H, Ruser R, Schilling R, Loftfield N, Munch JC, Kaiser EA, Beese F. 2002. N2O and CH4 fluxes in potato fields: Automated measurement, management effects and temporal variation. Geoderma 105(3-4):307-25.

IPCC. 2013. Climate change 2013: The physical science basis. contribution of working group 1 to the fifth assessment report of the intergovernmental panel on climate change. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

Isbell RF. 2002. Australian Soil Classification, Revised Edition. Collingwood, Vic, Australia: CSIRO Publishing.

Kennedy T, Decock C, Six J. 2013. Assessing drivers of N2O production in California tomato cropping systems. Science of the Total Environment 465(0):36-47.

Livesley SJ, Grover S, Hutley LB, Jamali H, Butterbach-Bahl K, Fest B, Beringer J, Arndt SK. 2011. Seasonal variation and fire effects on CH4, N2O and CO2 exchange in savanna soils of northern Australia. Agricultural and forest Meteorology 151(11):1440-52.

Liu C, Zheng X, Zhou Z, Han S, Wang Y, Wang K, Wang K, Liang W, Li M, Chen D, Yang Z. 2010. Nitrous oxide and nitric oxide emissions from an irrigated cotton field in northern China. Plant and Soil 332(1):123-134.

Luo J, Hoogendoorn C, van der Weerden T, Saggar S, de Klein C, Giltrap D, Rollo M, Rys G. 2013. Nitrous oxide emissions from grazed hill land in New Zealand. Agriculture, Ecosystems and Environment 181:58-68.

Maljanen M, Martikainen PJ, Aaltonen H, Silvola J. 2002. Short-term variation in fluxes of carbon dioxide, nitrous oxide and methane in cultivated and forested organic boreal soils. Soil Biology and Biochemistry 34(5):577-84.

Morris SG, Kimber SWL, Grace P, Van Zwieten L. 2013. Improving the statistical preparation for measuring soil N2O flux by closed chamber. Science of the Total Environment 465:166-72.

Mu ZJ, Huang AY, Ni JP, Li JQ, Liu YY, Shi S, Xie DT, Hatano R. 2013. Soil greenhouse gas fluxes and net global warming potential from intensively cultivated vegetable fields in southwestern China. Journal of Soil Science and Plant Nutrition 13(3):566-78.

Parkin TB. 2008. Effect of sampling frequency on estimates of cumulative nitrous oxide emissions. Journal of Environmental Quality 37(4):1390-5.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Ravishankara AR, Daniel JS, Portmann RW. 2009. Nitrous oxide (N2O): The dominant ozone-depleting substance emitted in the 21st century. Science 326(5949):123-5.

Rowlings DW, Grace PR, Scheer C, Liu S. 2015. Rainfall variability drives interannual variation in N2O emissions from a humid, subtropical pasture. Science of the Total Environment 512-513:8-18.

Savage K, Phillips R, Davidson E. 2014. High temporal frequency measurements of greenhouse gas emissions from soils. Biogeosciences 11(10):2709-20.

Scheer C, Grace PR, Rowlings DW, Payero J. 2012. Nitrous oxide emissions from irrigated wheat in Australia: Impact of irrigation management. Plant and Soil 359(1-2):351-62.

Scheer C, Rowlings DW, Firrel M, Deuter P, Morris S, Grace PR. 2014. Impact of nitrification inhibitor (DMPP) on soil nitrous oxide emissions from an intensive broccoli production system in sub-tropical Australia. Soil Biology and Biochemistry 77:243-51.

Smith K A, Ball T, Conen F, Dobbie KE, Massheder J, Rey A. 2003. Exchange of greenhouse gases between soil and atmosphere: Interactions of soil physical factors and biological processes. European Journal of Soil Science, 54(4):779-791.

Smith KA and Dobbie K. 2001. The impact of sampling frequency and sampling times on chamber-based measurements of N2O emissions from fertilized soils. Global Change Biology 7(8):933-45.

Smith KA, Dobbie KE, Thorman R, Watson JC, Chadwick DR, Yamulki S, Ball, BC. 2012. The effect of N fertilizer forms on nitrous oxide emissions from UK arable land and grassland. Nutrient Cycling in Agroecosystems. 93:127-149.

Syakila A and Kroeze C. 2011. The global nitrous oxide budget revisited. Greenhouse Gas Measurement and Management 1(1):17-26.

Van Der Weerden TJ, Clough TJ, Styles TM. 2013. Using near-continuous measurements of N2O emission from urine-affected soil to guide manual gas sampling regimes. New Zealand Journal of Agricultural Research 56(1):60-76.

Wang W, Dalal RC, Reeves SH, Butterbach-Bahl K, Kiese R. 2011. Greenhouse gas fluxes from an Australian subtropical cropland under long-term contrasting management regimes. Global Change Biology 17(10):3089-101.

Williams DL, Ineson P, Coward PA. 1999. Temporal variations in nitrous oxide fluxes from urine-affected grassland. Soil Biology and Biochemistry 31(5):779-88.

Zhang Y, Mu Y, Zhou Y, Liu J, Zhang C. 2014. Nitrous oxide emissions from maize-wheat field during 4 successive years in the north China plain. Biogeosciences 11(7):1717-26.

Yao Z, Zheng X, Xie B, Liu C, Mei B, Dong H, Butterbach-Bahl K, Zhu J. 2009. Comparison of manual and automated chambers for field measurements of N2O, CH4, CO2 fluxes from cultivated land. Atmospheric Environment 43(11):1888-96.