Optimum Sampling Time and Frequency for Measuring N₂O Emissions from a Rain-fed Cereal Cropping System

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Abstract

Annual cumulative nitrous oxide (N₂O) 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 N₂O 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 N₂O emissions were subsampled from the dataset to simulate different sampling frequencies, including pre-set and rainfall-based scenarios. Annual cumulative N₂O 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 N₂O 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 ($\pm 4\%$ error) of annual cumulative N₂O 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 ($\pm 5\%$ 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 N₂O emissions satisfactorily when timed appropriately.

1. Introduction

Nitrous Oxide (N₂O) is a potent greenhouse gas with a global warming potential 298 times that of carbon dioxide (IPCC, 2013). N₂O is also the largest ozone depleter in the atmosphere (Ravishankara et al., 2009). The concentration of N₂O 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 N₂O 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 (ON), 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 N₂O 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 N₂O emissions under ON 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 (N₂O: 0.5 ppm, CH₄: 2 ppm and CO₂ 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 N₂ 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

N₂O 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 N₂O emissions measured at 144-minute intervals over a 24 hr period. Some daily means were calculated from fewer emission values as those measurements with R²<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 N₂O emissions for assessing other less frequent sampling schedules.

To assess diurnal variability in N₂O 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 N₂O-N ha⁻¹ d⁻¹) were not included, with the remaining categorised as either low emission (2-20 g N₂O-N ha⁻¹ d⁻¹) or high emission (>20 g N₂O-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 N₂O emission rate (E_d), the N₂O 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 N₂O 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 N₂O 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 N₂O 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
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*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 N₂O emissions as the benchmark. The deviation (D_s) of the simulated (E_s) from the 'actual' (E_a) annual cumulative N₂O 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 N₂O Emissions

Over the three-year study period, N₂O emissions exhibited significant daily, seasonal and yearly variation (Fig. 1). Large peaks of N₂O emissions (>20 g N₂O-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 N₂O-N ha⁻¹ yr⁻¹, 1267 ± 201 g N₂O-N ha⁻¹ yr⁻¹, and 1941 ± 251 g N₂O-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 N₂O-N ha⁻¹ yr⁻¹, 1683 ± 1320 g N₂O-N ha⁻¹ yr⁻¹ and 652 ± 267 g N₂O-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 $\pm$ 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 $90 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ 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 ($>20 \text{ g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$) 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: $>20 \text{ g N}_2\text{O-N ha}^{-1} \text{d}^{-1}$) and low emission days (b: $2-20 \text{ g N}_2\text{O-N ha}^{-1} \text{d}^{-1}$). 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 N₂O emissions and the N₂O emissions estimated using measurements during the period from 09:00 to 12:00.

3.3. Impacts of Sampling Frequencies on Annual Cumulative N₂O Emissions

There was no consistent trend of over- or underestimation of the ‘actual’ annual cumulative N₂O emissions for a specific intermittent sampling schedule (Fig. 4). The potential outcomes were generally spread around the ‘actual’ annual cumulative N₂O 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 N₂O-N ha⁻¹ yr⁻¹. While the smallest variation, averaged across years, occurred in the Weekly + Triweekly sampling schedule with 33 g N₂O-N ha⁻¹ yr⁻¹. Largest variations across all sampling schedules occurred in 2007-2008 (28 – 807 g N₂O-N ha⁻¹ yr⁻¹), while the smallest were in 2006-2007 (15 – 80 g N₂O-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 $<\pm 7\%$ in all years, while the Weekly + Biweekly and Fortnightly + Biweekly schedules appeared less satisfactory in 2007-2008 with the errors reaching $\pm 10\%$.

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 ($>20 \text{ g } N_2O\text{-N ha}^{-1} \text{ d}^{-1}$). 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 N₂O emissions, however it does not always exert influence on N₂O 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 N₂O 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 N₂O 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 N₂O emission, instead theorising that it could also be temperature-induced changes in the solubility of N₂O in soil water. These hypotheses could explain the diurnal cycles in N₂O 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 N₂O emissions is not consistent through time, its presence on any particular day means manual gas sampling has the potential to bias cumulative N₂O 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 N₂O 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 N₂O 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 N₂O 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 N₂O 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 N₂O 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 N₂O emission can be satisfactorily estimated by intermittent samplings. Using long-term sub-daily N₂O emission data, this study has assessed the most appropriate time of day to sample N₂O 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 N₂O 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 N₂O emissions, with $\pm 4\%$ error, although its high sampling intensity was undesirable. weekly sampling with triweekly sampling in the two weeks following rainfall events had similar accuracy ($\pm 5\%$ 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.

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