Quantifying Nitrous Oxide Emissions from Sugarcane Cropping Systems: Optimum Sampling Time and Frequency

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Abstract

Nitrous oxide (N₂O) emissions from soil are often measured using the manual static chamber method. Manual gas sampling is labour intensive, so a minimal sampling frequency that maintains the accuracy of measurements would be desirable. However, the high temporal (diurnal, daily and seasonal) variabilities of N₂O emissions can compromise the accuracy of measurements if not addressed adequately when formulating a sampling schedule. Assessments of sampling strategies to date have focussed on relatively low emission systems with high episodicity, where a small number of the highest emission peaks can be critically important in the measurement of whole season cumulative emissions. Using year-long, automated sub-daily N₂O measurements from three fertilised sugarcane fields, we undertook an evaluation of the optimum gas sampling strategies in high emission systems with relatively long emission episodes. The results indicated that sampling in the morning between 09:00-12:00, when soil temperature was generally close to the daily average, best approximated the daily mean N₂O emission within 4-7% of the 'actual' daily emissions measured by automated sampling. Weekly sampling with biweekly sampling for one week after > 20 mm of rainfall was the recommended sampling regime. It resulted in no extreme (>20%) deviations from the 'actuals', had a high probability of estimating the annual cumulative emissions within 10% precision, with practicable sampling numbers in comparison to other sampling regimes. This provides robust and useful guidance for manual gas sampling in sugarcane cropping systems, although further adjustments by the operators in terms of expected measurement accuracy and resource availability are encouraged. By implementing these sampling strategies together, labour inputs and errors in measured cumulative N₂O emissions can be minimised. Further research is needed to quantify the spatial variability of N₂O emissions within sugarcane cropping and to develop techniques for effectively addressing both spatial and temporal variabilities simultaneously.

1. Introduction

Nitrous oxide (N₂O) is a potent greenhouse gas, with a global warming potential 298 times that of carbon dioxide and is also the main contributor to the depletion of ozone in the stratosphere (Ravishankara *et al.* 2009). Increases in the concentration of atmospheric N₂O have been largely attributed to increases in usage of nitrogenous fertilisers and organic amendments applied to soils (Davidson, 2009). Soil microbes utilise mineral nitrogen (NH₄⁺ and NO₃⁻) generated from the nitrogenous inputs during the nitrification and denitrification processes and emit N₂O as a by-product or intermediate. Loss of nitrogen (N) as N₂O, which is often accompanied by dinitrogen loss during denitrification, is also an economic cost, either as a direct loss of purchased fertiliser N or as a loss in potential yield. Therefore there are both environmental and economic interests in measuring

N₂O emission from cropped soils, with the ultimate goal of reducing N₂O losses and increasing fertiliser N use by crops.

Measurements of N₂O emissions from soils, until recently, relied heavily on the manual static chamber method (Parkin and Venterea, 2010; Dalal *et al.* 2003). This method generally employs a collared base inserted into the soil, with an air tight enclosure placed on the collar only during sampling. Using a syringe, a series of samples are extracted from the enclosed headspace over a period of time (~0.5 to 2 hours), injected into vials and then transported to a laboratory for analysis. Benefits of this method for agricultural field trials include the ability to accommodate large numbers of replications, low initial setup costs and simple deployment. Due to the time required to sample and often the travel time to and from field sites, the manual static chamber method is labour and time intensive, therefore limiting the frequency of sampling. Furthermore, the majority of recent N₂O studies employing manual chambers have taken measurements during mid-morning and assumed this is an appropriate time of day to sample (Bell *et al.* 2015; Hinton *et al.* 2015; Yang *et al.* 2015; Zhou *et al.* 2015). This is even though some studies have reported large underestimations of up to 43% of the mean seasonal N₂O emissions when sampling occurred mid-morning (Scheer *et al.* 2012). Infrequent sampling has the potential to overlook both diurnal variability and day to day variability, and subsequently is considered one of the major disadvantages of this method.

In contrast to manual static chambers, automated static chamber systems allow measurement of emissions at a sub-daily frequency. The automated sampling systems generally conduct analysis in-situ, and thus are less labour- and time-consuming. However, they are often limited to a set number of chambers and have high setup costs; their reach within a field trial is constrained by the length of sample lines; and site selection is limited by power availability.

The sub-daily data collected by automated chamber systems can provide a basis on which to assess optimum sampling strategies for manual static chambers, and to reduce any biases and inaccuracies caused by discontinuous samplings. The majority of previous assessments from sub-daily data have evaluated fixed sampling intervals from 1-31 days (Morris *et al.* 2013; Liu *et al.* 2010; Parkin, 2008). However, strategic samplings based around high emission events such as rainfall and fertilisation, may offer more accurate estimations of cumulative N₂O emissions with reduced labour inputs in field studies (Barton *et al.* 2015; Reeves and Wang, 2015). Therefore an assessment of event based sampling strategies is required to give certainty to, and provide improvements for, future sampling regimes.

Sugarcane is cropped on over 28.7 million hectares of land globally (FAO, 2015). Sugarcane farms are all located in wet and warm tropics and subtropics and generally emit large amounts of N₂O, in the range between 1.5 and 45.9 kg N₂O-N/ha/year (Soares *et al.* 2015; Wang *et al.* 2016; Denmead *et al.* 2011). Therefore accurate measurement of N₂O emissions from sugarcane is of importance. Strategic rainfall-based sampling regimes have previously been assessed in a wheat cropping system (Reeves and Wang, 2015), an unfertilised pasture and rainforest (Rowlings *et al.* 2015) in Australia and a grazed pasture in New Zealand (Van Der Weerden *et al.* 2013). Conclusions from previous studies have been contrasting with daily, three times a week and various different strategic schedules being recommended, which is evidence that in different systems different sampling strategies may be required (Barton *et al.* 2015; Reeves and Wang, 2015; Van Der Weerden *et al.* 2013). Furthermore, the most comprehensive assessment of fixed sampling schedules to date, at nine sites, did not include any sugarcane cropped sites and indeed advocated further assessments in different agricultural and environmental systems (Barton *et al.* 2015). The lack of representation of sugarcane cropping systems in previous sampling frequency assessments needs to be addressed, as N₂O emissions from sugarcane cropped soils exhibit lower episodicity, which could impact the sampling frequency required. The large nitrogen fertiliser inputs (typically between 120 and 230

kg/ha in Australia) shortly before the wet and warm summer season cause a distinct prolonged high N₂O emission period over spring and summer. This would justify implementation of a focused sampling strategy around high rainfall periods, that can reduce the overall number of sampling days with minimal loss of accuracy in measured annual N₂O emissions.

With this in mind, the objective of the study is to determine the most temporally efficient and effective sampling strategies when using static chambers to quantitatively measure N₂O emissions from sugarcane fields. This study uses year-long sub-daily data from three sugarcane sites to assess (i) the time of day which best approximates the daily mean N₂O emission rate, and (ii) simulate a number of different sampling regimes, including both fixed and rainfall based, to ascertain the most effective and efficient sampling frequency to estimate annual cumulative N₂O emissions.

2. Methods

2.1. Experimental sites and treatments

Sub-daily N₂O emissions were measured at three sites located on the eastern coastline of Australia, ranging from southern to northern Queensland (Fig. 1). All experiments were established as a randomised block design with multiple treatments, consisting of different fertiliser rates, fertiliser types, and management practices (Wang *et al.* 2015; Wang *et al.* 2014; Wang *et al.* 2012). At each site, due to a limited number of automated chambers and length of sampling lines, only two treatments were measured for N₂O emissions using an automatic sampling system. One treatment had no fertiliser addition and the other was a treatment with N fertiliser added at recommended rates as urea (140 -150 kg N/ha). For the purposes of this study, only the fertilised treatment was used, as it had higher emissions, which would elucidate diurnal and daily changes better than the nil fertiliser treatment.

Fig. 1. Location of sugarcane study sites in Queensland, Australia. Inset graphs are long term averages (1981 – 2010) of monthly rainfall (column) and mean temperature (line).

Site 1 was located on a sugarcane farm in the sub-tropics at Bundaberg, Queensland (24°57' S, 152°20' E). After a five year cane cycle, and a 12 month period of fallow, sugarcane was planted in single rows on raised beds. Urea fertiliser was added at a rate of 25 kg N/ha at planting on 2 September 2013, with an additional 120 kg N/ha side-dressed and buried to about 10 cm approximately two months later. Measurements of N₂O emissions from 4 September 2013 to 15 September 2014 were used in the present study, totalling 376 days. The crop was harvested on 16 September 2014. Green cane trash blanketing (GCTB), the retention of crop residues on the soil surface after harvest, was practised from 2007-2012. The long-term (1981-2010) annual mean temperature is 21.8 °C (Bundaberg Aero Station, the Bureau of Meteorology, Australia), with the lowest monthly mean temperature in July (16.6 °C) and the highest in January (26.1 °C) (Fig. 1). Mean annual rainfall is 967 mm, with ca. 42% of rainfall received during summer from December to February. The soil is classified as a Redoxic Hydrosol (Isbell, 2002) under the Australian Soil Classification (ASC) and a Gleysol (IUSS Working Group WRB, 2014) by the World Reference Base soil classification (WRB), with a loamy sand in the top 30cm and a light mottled clay from 50-150cm. The site was irrigated on 30 October 2013 (79 mm) and 29 December 2013 (70 mm). A more detailed description of this experiment was given by Wang *et al.* (2015).

The experiment at Site 2 was established near Mackay (21°11' S, 149°06' E) in sub-tropical central Queensland (Wang *et al.* 2012). Sugarcane was planted on 26-27 July 2010, following more than eight months of fallow, with a single row in the middle of each raised bed. Approximately three months after planting, urea fertiliser was applied subsurface in a band on either side of the sugarcane row, at a rate of 150 kg N/ha. Measurements of N₂O emission began on 27 July 2010 after planting and finished on 19 July 2011, totalling 357 days. Harvest followed shortly after on 20 November 2011. Green cane trash blanketing had been practised for several years prior to trial establishment. Annual mean temperature (1981-2010) in this area is 22.4 °C and the mean annual rainfall is 1612 mm (Te Kowai Experimental Station, the Bureau of Meteorology, Australia) (Fig. 1). The lowest monthly mean temperature is in July (16.9 °C), while the highest is in January (26.7 °C). 50% of annual rainfall occurs in the summer season from December to February. The soil is classified as a Chromosol (Isbell, 2002) by the ASC and a Lixisol (IUSS Working Group WRB, 2014) by the WRB, with a sandy loam layer in the 0-35 cm, underlain by sandy clay between 35 and 100 cm depth.

The third experimental site was located in the wet tropics (18°42' S, 146°08' E), near the town of Ingham, Queensland (Wang *et al.* 2014). Unlike the other two sites in this study, which were conducted with plant cane (i.e. sugarcane was planted in the year of measurement), this experimental site consisted of a 1st ratoon sugarcane crop (i.e. sugarcane was allowed to regrow following the harvest of the plant cane crop). The crop was fertilised subsurface (ca. 10cm) in the middle of the pre-existing cane row with urea at a rate of 140 kg N/ha on 4 October 2012. Following fertilisation, N₂O measurements began on 5 October 2012 and finished on 8 October 2013, after harvest on 6 August 2013, totalling 368 days. GCTB had been practised on this farm since 1985. Long-term (1981-2010) annual mean temperature is 24.2 °C (Ingham Composite Station, the Bureau of Meteorology, Australia), with the lowest monthly mean temperature in July (19.4 °C) and the highest in January (27.7 °C) (Fig. 1). Mean annual rainfall is 2055 mm, with c.a. 50% received during summer from December to February. The soil is classified as a Redoxic Hydrosol (Isbell, 2002) by the ASC and a Gleysol (IUSS Working Group WRB, 2014) by the WRB, with a silty clay loam in the 0-25 cm layer, underlain by a silty clay layer at 25-60 cm depth.

2.2. Nitrous oxide measurements

Emissions of N₂O were measured using an automated chamber system (Wang *et al.* 2011) with either 9 (Site 2 and 3) or 12 (Site 1) chambers. The chambers consisted of a square base (50 cm x 50 cm), which was inserted approximately 10 cm into the soil, and a removable metal framed perspex cover box with opening/closing lid. Each chamber had an associated spare base inserted into the soil, so that the cover boxes could be moved between bases intermittently to minimise modification of the micro climate (e.g. possible reduced rainfall) inside the chamber. In each treatment plot, one chamber was placed in the inter-row space and one chamber was placed on the raised bed area covering where the fertiliser was placed, except at Site 3 where two chambers were placed on the bed and one in the inter-row space for one plot. At Site 1 three replicated plots were monitored, while at Sites 2 and 3 two replicated plots were monitored.

Each chamber was connected to a sampling system, which extracted air from the headspace of the chamber, and delivered it via teflon tubing (diameter 1/16", ~ 1.58 mm) to a gas chromatograph (SRI 8610C, SRI Instruments, CA, Torrance, USA) equipped with an electron capture detector (ECD) to measure N₂O concentration. The sample passed through an ascarite pre-column to remove carbon dioxide, before being separated on a packed column (Site 1: Haye Sep D; Site 2 and 3: Haye Sep N; Mesh 60/80; diameter 1/8" ~ 3.3 mm; length 6 ft, ~ 182 mm) using N₂ as the carrier gas. Column oven and ECD temperatures were 50°C and 330°C, respectively.

Chambers were closed as a set of four at Site 1 and in a set of three at Site 2 and 3. Samples were extracted from chamber headspaces at 3 minute intervals, with a single chamber being sampled four times over a period of 48 minutes at Sites 2 and 3, and over a period of 60 minutes at Site 1. A calibration gas containing 5 µL/L N₂O (Air Liquide Australia Ltd.) was analysed after every three headspace samples at Sites 2 and 3, and after every four headspace samples at Site 1, to enable the N₂O concentration to be calculated.

Hourly air temperature and rainfall were measured using an onsite weather station (CR200, Campbell Scientific, Australia) at Sites 1 and 3. Rainfall data at Site 2 was drawn from a weather station (#033047; 2.1 km away) administered by the Bureau of Meteorology, however hourly air temperature data was not available on site or at nearby weather stations for the experimental period. Soil temperature, at 5-7 cm depth, was measured at 1 hour intervals using temperature probes (Measurement Engineering Australia, S.A., Australia) at all sites.

2.3. Data Analysis

A detailed description of the methods used for data analyses were given in Reeves and Wang (2015). N₂O emissions were calculated from the measured increase in gas concentration, across four air samples extracted from the chamber headspace at regular intervals, using linear regression. Any set of four samples with a R² value of <0.80 were excluded from the dataset, as the concentration increase was considered nonlinear. The remaining emissions data were then averaged to create a mean daily N₂O emission for each chamber. The potential number of emission values measured for each chamber was 8 per day for Site 1 and 10 per day for Sites 2 and 3. To calculate the mean daily N₂O emission per plot, the mean daily N₂O emission from each position (bed and inter-row) was weight averaged depending on the proportion of bed and inter-row space. Annual cumulative N₂O emissions were calculated as the sum of all daily emission means. For the purposes of this study, these calculated cumulative emissions will be referred to as the 'actual' annual cumulative emissions, and are the benchmark with which other sampling schedules are compared to. In situations where emissions data within the defined measurement periods were missing due to instrument breakdown, removal of chambers, extreme weather (e.g. cyclone) or management

practices (e.g. fertilisation application, herbicide spraying), the data for missing days were interpolated from measured data either side of that time. If data were missing in the lead up to a significant rainfall event, emissions from the last measured day before the missing data were extrapolated up to the rainfall event to minimise overestimating emissions.

Episodicity (EI) of emissions was calculated using an equation modified from Smith and Hunt (1978):

$$EI = \left(\frac{ED}{D} \right) * 100\% \quad (1)$$

where ED is the episode days as the number of those days with the highest emissions, which when summed equal 40% of the total emissions over the entire time period; D is, the total number of days (D) over the entire time period. The lower the EI, the higher the episodicity is.

Diurnal variability in emissions was examined by partitioning the original sub-daily dataset into 8 time periods, of three hours duration each, depending on when a gas sample was taken. As individual emission rates were calculated over a period of 60 or 48 mins, the time at the middle of the measurement period was used to assign each value to a specific time of day. Only chambers located on the bed were used for the diurnal analysis, as they generally had larger emissions, due to N fertilisation. As such there were three, two and three replicates for Sites 1, 2 and 3, respectively. Days with ≥ 3 missing measurements were excluded from further analysis. If only one or two measurements were missing, the missing values were estimated by linear interpolation. The days when rainfall (> 5 mm) occurred were excluded, and upon visual inspection of the remaining data, days without clear trends in emissions were excluded. These days may have had other regulating factors obscuring diurnal variability (e.g. soil moisture change or nitrogen limitation). 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) were assessed as follows:

$$D_t = \left(\frac{E_t - E_d}{E_d} \right) * 100\% \quad (2)$$

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 daily mean emission rate (E_d), was calculated as the average of all time period emission rates in a 24 hour period.

To assess the effectiveness of intermittent sampling schedules in accurately measuring the cumulative emissions over the experimental time period, the full set of daily mean N_2O emissions for each site was systematically subsampled according to the sampling schedules outlined in Table 1. The time period that emissions were closest to the daily mean, as determined from the diurnal analysis, were also systematically subsampled to compare against outcomes from the daily mean N_2O emissions, and to better represent a realistic manual gas sampling regime. Both fixed (i.e. Triweekly, Biweekly, Weekly and Fortnightly) and rainfall-based (i.e. fixed Weekly and Fortnightly with Biweekly or Triweekly sampling after rainfall) sampling schedules were included. More intensive gas sampling schedules after N fertiliser application were not assessed because no substantial increases in N_2O emissions were observed immediately after fertilisation until > 20 mm of rainfall was received (Fig. 2). For the rainfall-based samplings, the increased sampling time period (one or two weeks) started the day after daily rainfall of > 20 mm was received. If a > 20 mm rainfall event occurred within the one- or two-week period following a previous > 20 mm rainfall event, the one- or two-week period of intense sampling was recounted from the later event. Also for rainfall-based sampling schedules, in the lead up to a rainfall event of > 20 mm, the last sampling before the

rainfall was extrapolated up to the event. From the subsampled dataset, cumulative emissions were then calculated using linear interpolation between intermittent sampling days.

The accuracy of sampling schedules was assessed using the deviation of the estimated cumulative emissions (E_s) from the 'actual' cumulative emissions (E_a):

$$D_s = \left(\frac{E_s - E_a}{E_a} \right) * 100\% \quad (3)$$

Where the deviation (D_s) indicates, as a percentage of E_a , how close E_s estimated E_a for a single sampling schedule outcome. The data distribution was non-normal ($P < 0.001$) based on the Shapiro-Wilk normality test. Therefore to compare different sampling schedule outcomes, the non-parametric Kruskal-Wallis ANOVA was used on the non-transformed data. Because of unequal sample sizes, this was followed by Dunn's post hoc pairwise multiple comparison procedure, with Bonferroni correction to control false positive results. 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:

$$P_a = \left(\frac{N_a}{N} \right) * 100\% \quad (4)$$

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

Table 1: Simulated sampling schedules

Sampling Schedule	Options	Number of sampling days			Number of Outcomes plot ⁻¹ year ⁻¹
		Site 1	Site 2	Site 3	
Triweekly	Monday, Wednesday & Friday.	162	153	158	1
Biweekly	Monday & Thursday; or Tuesday & Friday; or Monday & Friday.	108	102	106	3
Weekly	Monday; or Tuesday; or Wednesday; or Thursday; or Friday.	54	51	53	5
Fortnightly	Fortnightly cycle of Monday; or Tuesday; or Wednesday; or Thursday; or Friday.	27	26	26	10
Weekly + 1 Triweekly	As per weekly sampling, but triweekly for 1 week after >20 mm daily rainfall.	77	101	73	5
Weekly + 1 Biweekly	As per weekly sampling, but biweekly for 1 week after >20 mm daily rainfall.	65	77	61	15

Weekly + 2 Triweekly	As per weekly sampling, but triweekly for 2 weeks after >20 mm daily rainfall.	97	116	87	5
Weekly + 2 Biweekly	As per weekly sampling, but biweekly for 2 weeks after >20 mm daily rainfall.	76	84	70	15
Fortnightly + 1 Triweekly	As per fortnightly sampling, but triweekly for 1 week after >20 mm daily rainfall.	54	88	62	10
Fortnightly + 1 Biweekly	As per fortnightly sampling, but biweekly for 1 week after >20 mm daily rainfall.	44	63	41	30
Fortnightly + 2 Triweekly	As per fortnightly sampling, but triweekly for 2 weeks after >20 mm daily rainfall.	80	107	70	10
Fortnightly + 2 Biweekly	As per fortnightly sampling, but biweekly for 2 weeks after >20 mm daily rainfall.	59	75	53	30

308

309 3. Results

310 3.1. Dynamics of N₂O emissions

311 Cumulative N₂O emissions, calculated from all measurements, were 5.6 ± 1.0 , 15.3 ± 2.4 and $18.2 \pm$
312 4.5 kg N/ha, for Sites 1, 2 and 3, respectively. At each site, the majority of emissions occurred before
313 the end of March (i.e. the first month in autumn), with > 60% of total emissions for Site 1 and > 80 %
314 for Sites 2 and 3 occurring during this time (Fig. 2). The episodicity of emissions over the whole
315 measurement period was highest at Site 1 (6.6%), followed by Site 2 (7.3%) and Site 3 (7.4%). Large
316 increases in daily emissions and therefore the rate of cumulative emissions were generally related to
317 rainfall events, but not all rainfall events resulted in increases in emissions. This is most obvious at
318 Site 2 from February to April (weeks 29 to 37) where continuous rainfall did not increase cumulative
319 emissions, most likely due to water logged conditions (Fig. 2b). Total rainfall over the measurement
320 period was 916 mm, 3541 mm and 1662 mm for Sites 1, 2 and 3, respectively. Sites 1 and 3 had less
321 rainfall than the long term average, but Site 2 had 220% more rainfall than the long term average.
322 Site 2 also had 49 daily rainfall events of > 20mm, compared to < 20 such events at Sites 1 and 3.

324

325 Fig. 2. Dynamics of rainfall and daily N₂O emissions at each site. P and F indicate when planting and
326 fertiliser application occurred, respectively.

327 3.2. Impact of sampling time on estimation of mean daily emissions

328 Up to 42% of days at each site generated diurnal trends, and were largely evident during high
329 emission periods, indicating sampling time of day was more important during high than low emission
330 periods. At all three sites, N₂O emissions during these periods displayed obvious sinusoidal patterns
331 (Fig. 3). A general trend of overestimation of mean daily emissions was observed in the afternoon

and early evening, while an underestimation was evident late night to early morning. The peak overestimation in emissions occurred between 12:00 and 18:00, and ranged from 1% to 20%. Peak underestimation occurred between 06:00 and 09:00 at each site and ranged from -5% to -20%. Between 09:00-12:00 and 12:00-15:00, there was a pronounced change in the estimation accuracy from a minor underestimation to a marked overestimation of the daily mean emission. Overall, the three hours prior to midnight (21:00-24:00) and late morning-midday (09:00-12:00) provided the best approximation of the daily mean at all sites.

Peaks and troughs in daily air and soil temperature generally coincided with the peaks and troughs in emissions (Fig. 3). However, the peak in emissions at Site 1 lagged from the peak in air and soil temperature by approximately 2-3 hours. In contrast, the peak emissions at Site 3 occurred ahead of the peak in soil temperature by 2-3 hours. The air temperature from 08:00-09:00 in the morning and from 19:00-20:00 at night were close to the daily mean temperature. The soil temperature from 09:00-11:00 in the morning and from 19:00 to 22:00 at night were close to the mean daily soil temperature, which coincided with daily mean N₂O emissions more closely compared to air temperature.

Fig. 3. Diurnal variation in N_2O emissions, as an average of multiple days, from the fertilised bed position at three sugarcane sites. Vertical bars are standard error of multiple days. Dotted lines in (a) and (c) are average air temperature at different times of day over the experimental period. No hourly air temperature data were available for (b). Solid black lines are average soil temperatures at different times of day over the experimental period. The soil temperature was measured at 5-7cm depth.

3.3. Impact of sampling frequency on cumulative emissions estimation

The absolute deviations of cumulative N_2O emissions from the 'actuals' were generally similar or larger when calculated using data collected between 09:00-12:00 (the period when N_2O emissions were closest to the daily mean N_2O emissions), compared to those based on the daily mean N_2O emissions. Furthermore, the range (minimum to maximum) was generally larger when data between 09:00-12:00 was sub-sampled (Fig. 4.). This indicates that more variability is introduced

from the diurnal fluctuation in N₂O emissions. As manual chamber samplings are usually conducted at only one time per day, the comparison of different sampling schedules hereafter will be based on the 09:00-12:00 data only, so as to better represent a real world manual sampling scenario. This method consequently encompasses the effect of diurnal variability and day to day variability in the assessment of cumulative emissions.

The spatial variability in emissions as quantified by the coefficient of variation (CV) was 29%, 23%, and 35% for Sites 1, 2 and 3 respectively. The variability (CV) of the Fortnightly sampling schedule consistently exceeded the spatial variability at each site, and therefore cannot be considered as a suitable sampling schedule (Fig 5.).

The average annual N₂O emissions across all outcomes for each simulated sampling schedule were mostly within 5% of the 'actuals'. Higher absolute deviation values were obtained for the fixed Weekly (7±5%) and Fortnightly samplings (18±14%), Fortnightly sampling with increased frequency for one week after rainfall (+ Triweekly 8±4%, + Biweekly 10±6%) and Fortnightly with biweekly sampling for two weeks following rainfall (6±5%). The fixed Triweekly and Biweekly schedules performed at a similar accuracy of 4±3% and 5±3%, respectively. The fixed schedules generally followed a trend of increased accuracy (i.e., decreased absolute value of deviation) with increased sampling frequency (Triweekly > Biweekly > Weekly > Fortnightly). The rainfall-based sampling schedules all improved accuracy compared to their associated fixed sampling schedules; however, the extent of the improvement varied across sites. For example in the case of the Fortnightly schedule, increasing sampling to Biweekly or Triweekly after rainfall events decreased the absolute deviation from ca. 16% to 7% at Site 1 and 3, and from ca. 24% to 8% at Site 2. The resulting decrease in deviation was similar, whether sampling was increased to Biweekly or Triweekly after rainfall, but outcomes from the +Biweekly schedules had higher variation between different sampling days compared to the +Triweekly schedules (Fig. 5). Following rainfall events, increasing sampling frequency for two weeks in a Fortnightly schedule improved the accuracy more than only increasing sampling frequency for one week. In contrast, whether sampling was increased for either one or two weeks following rainfall in a Weekly schedule, the accuracy improvements were similar.

Kruskal-Wallis ANOVAs indicated significant differences in absolute deviations between schedules at each site and across all three sites ($P < 0.001$). The sampling schedule with the smallest deviation from the actual annual cumulative emissions differed at different sites. At Site 1, it was Weekly + 2 Triweekly (3%); at Site 2, Triweekly (4%); and at Site 3, Weekly + 1 Triweekly (2%). However, it should be noted that in each case these sampling schedules were only marginally better than some other sampling schedules. Overall, the Fortnightly, Fortnightly +1 Biweekly and Fortnightly + 1 Triweekly schedules were significantly less accurate than other sampling schedules ($P < 0.05$), and therefore are not recommended.

There was generally no consistent over- or underestimation of cumulative emissions, thus the variation in sampling outcomes for a given schedule is evidence of the risk associated with using that sampling schedule. Those sampling schedules with higher variation have inherently higher risk that a particular chosen sampling day (i.e. Monday, Tuesday etc.) will inaccurately estimate annual emissions. In contrast, those with lower variation have lower risk, as all chosen sampling options would produce similar estimation accuracies. Fixed Fortnightly and Weekly sampling schedules, as well as Fortnightly + 1 Biweekly had the largest variations (stdev) at each site. Furthermore, Weekly, Fortnightly, Fortnightly + 1 Biweekly, Fortnightly + 2 Triweekly and Fortnightly + 2 Biweekly had extreme outcomes at one or more sites that exceeded 20% deviation from the 'actuals', indicating an unacceptable impact on estimation accuracy if that sampling day was chosen and therefore are not recommended (Fig. 4.).

Overall, increasing gas sampling frequency after rainfall events increased the probability of estimating cumulative emissions within 10% for both Weekly and Fortnightly sampling schedules (Fig 6a.) Increasing from +Biweekly sampling after rainfall events to +Triweekly sampling increased the probability within 10% precision. A probability of 100% within 10% precision was achieved by Triweekly sampling and Weekly + 2 Triweekly sampling at all three sites. While overall, of the sampling schedules which haven't been excluded, a probability of >80% was achieved by Biweekly, Weekly + 1 Triweekly, Weekly + 1 Biweekly and Weekly + 2 Biweekly.

Ultimately, taking into account the overall accuracy, variability in accuracy, probability within 10% precision and individual excessive outcomes, the schedules which estimated the cumulative emissions best were fixed Triweekly and Biweekly samplings, as well as Weekly + Biweekly and Weekly + Triweekly samplings for one or two weeks after rainfall. Among them, the Weekly + 1 Biweekly schedule had the smallest number of sampling days (Table 1), which was on average a 57% reduction in sampling days compared to the most frequent Triweekly sampling.

Fig. 4. Box plots showing the deviation of outcomes of different sampling schedules from the 'actual' cumulative N_2O emissions calculated using daily mean emissions. The outcomes were annual cumulative N_2O emissions (kg N/ha) calculated from the emissions measured during 09:00-12:00. Box plots show median values (horizontal line), 25th and 75th percentile values (box outline) and minimum and maximum values (whiskers). Data normalised against cumulative emissions calculated from all sub-daily emissions data. Dotted line indicates zero, or no difference from the actual annual cumulative emissions. Light grey lines indicate 20% deviation from the actual annual cumulative emissions.

Fig. 5. The possible annual cumulative N₂O emissions from all sampling schedules, for replicated plots at each site. Outcomes were calculated from data collected between 09:00-12:00 am. Dotted lines represent the actual annual cumulative emissions for each replicate, calculated from all sub-daily emissions.

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 at each site. Probabilities were calculated from outcomes using data measured during 09:00-12:00. The overall (a) is aggregated data from all three sites.

4. Discussion

4.1. Diurnal variability in emissions and implication for sampling time of day

Diurnal variation in N₂O emissions was evident at all three sites and is consistent with the majority of previously observed trends in other environments. Peaks in emissions occurring during the afternoon have similarly been observed from a potato field (Hall *et al.* 2014), tropical grass field (Alves *et al.* 2012; Jantalia *et al.* 2008), bare fallow soil (Alves *et al.* 2012), cotton field (Liu *et al.* 2010) and wheat field (Reeves and Wang, 2015). With the diurnal fluctuations in emissions closely following the diurnal cycle of soil temperature, and to a lesser degree air temperature in this study, it is apparent that changes in temperature are driving N₂O emissions within a day. Large Q₁₀ temperature coefficients (i.e. the change in emission with a 10°C change in temperature) of more than 10, have been recorded for emissions of N₂O (Smith *et al.* 1998) and these are consistent with our understanding of the importance of temperature in driving diurnal variation in N₂O emissions. The change in temperature can affect the emission of N₂O by (i) increasing the rate of denitrification, and (ii) increasing the size of anaerobic zones that are conducive to denitrification (Smith *et al.* 2003).

To find the sampling time period which best approximates the mean daily emissions, the time period that corresponds to the mean daily air temperature has previously been recommended (Parkin and Venterea, 2010). However, the data in our studies demonstrated that the variation in soil temperature coincided more closely to the diurnal fluctuation of N₂O emission than air temperature (Fig. 3). As the production and emission of N₂O occur in the soil matrix, soil rather than air temperature directly influences microbial processes. This is an important distinction to make as peaks in air temperature may not occur at the same time as peaks in emissions, due to a time lag in the change in soil temperature at the depth where the N₂O production is occurring. Similarly, measuring soil temperature at a specific depth may not always coincide well with diurnal emission cycles if the depth measured is far away from the primary site of N₂O production or if there is a lag time between production and emission from the soil. Therefore, peaks in daily soil or air temperature may not exactly correspond to peaks in daily emissions as observed by Scheer *et al.* (2012).

At all three sugarcane sites, taking into account only daylight hours, gas sampling between 09:00 and 12:00 in the morning had the smallest deviation from the mean daily emissions, and is therefore recommended for gas sampling in sugarcane crops. Similar time periods for gas sampling have been suggested in other environments (Reeves and Wang, 2015; Savage *et al.* 2014; Van Der Weerden *et al.* 2013; Alves *et al.* 2012; Liu *et al.* 2010)

4.2. Daily variation in N₂O emissions and influence on gas sampling frequency

The annual data from three sugarcane cropping systems allowed comparison of the effectiveness of sampling schedules applied to different N₂O emission dynamics occurring under different edaphic and climatic conditions. Compared to a lower rainfall and lower emission cereal cropping system (Reeves and Wang, 2015), the overall accuracy of the intermittent sampling schedules was higher in the current study. The main reason for this difference is the lower episodicity of N₂O emissions in the sugarcane cropping systems compared to the low-rainfall cereal cropping system. Similar findings were asserted by Barton *et al.* (2015) that the episodicity of emissions controlled the minimum sampling frequency required. Further support for this theory is given by the fact that the episodicity was highest at Site 1, and therefore the coarser sampling schedules (i.e. fixed Weekly and Fortnightly) at Site 1 had the largest deviations from the 'actuals' in comparison to other sites. In systems dominated by low emissions with rare and short-lived high emission peaks, these peaks hold more weight in the calculation of cumulative emissions compared to those in systems where emission peaks occur more frequently and sustain over longer periods of time. Therefore,

inadequate sampling frequency has a higher chance to miss and/or over-extrapolate emission peaks in low emission systems than in high emission systems.

Overall, increasing the frequency of fixed sampling schedules increased the accuracy of estimated cumulative emissions in the order of Fortnightly < Weekly < Biweekly < Triweekly (Figs. 4 and 5) and decreased the variability in outcomes between the optional sampling days of the week (Fig. 4). This supports previous findings from a wheat cropping system and corn/soybean field (Reeves and Wang, 2015; Parkin, 2008).

To be effective, event-based sampling schedules should rely on an understanding of the effects of agronomic, soil and/or climatic factors on emissions of N₂O in a particular environment. It is well established that both soil temperature and soil oxygen content (through soil moisture) influence emissions and that agronomic practices such as fertiliser and/or residue inputs provide necessary substrates for microbial processes (Dalal et al., 2003). In this study, all three sites had crop residues retained on the surface, were planted/ratooned in spring, and fertilised shortly after. Therefore, N from fertiliser and C from residues were available to microbes during the subsequent warm and wet summer seasons. These conditions are conducive to N₂O production/emission and help explain why the majority of N₂O emissions occurred during the 4-6 months between fertilisation and the end of summer (Wang *et al.*, 2016). Coinciding with the majority of emissions, > 78% of rainfall over the measurement period occurred during this period. Thus, targeted sampling schedules around rainfall events could be more effective in efficiently using labour resources. This was backed up by improved accuracy in cumulative emissions estimates in comparison to fixed equivalents (e.g. fixed Fortnightly vs rainfall-based Fortnightly +Biweekly after rainfall). It should be noted that processing of data to reduce over-estimations prior to a rainfall event in rainfall-based sampling schedules, is vital to minimise inaccuracies in the calculated annual cumulative emissions. Similar findings were observed in a rain-fed winter crop system (Reeves and Wang, 2015). Also, Barton *et al.* (2015) reported no statistical difference between 'actuals' and intermittently measured N₂O emissions when strategic sampling, taking into account rainfall, temperature and/or time of year, was applied at three sites. However, contrasting data were reported by Rowlings *et al.* (2015), where a decreased accuracy was observed from a rainfall-based sampling schedule. The reasons for these discrepancies may be related to N₂O dynamics and/or data processing in different studies. If N₂O emissions are not moisture-limited then sampling around rainfall events may not improve accuracy in the measurement of cumulative emissions.

4.3. The need to compromise between accuracy and practicality

Although the accuracy of the emission estimates is important, the practicality of the sampling schedule in terms of time, labour and cost is also vital. Frequent sampling without achieving a substantial increase in accuracy, compared to a less frequent sampling schedule, would not be an efficient use of resources. Therefore, the number of samplings needed in any schedule should be examined against its' accuracy and resource requirement/availability. For example, the number of sampling days in the most intensive Triweekly sampling, could be reduced by a third in a Biweekly sampling with minimal effect on accuracy, indicating that the Biweekly sampling would be more efficient than Triweekly.

Event-based sampling schedules such as those with a higher frequency following a substantial rainfall have been suggested by Van Der Weerden *et al.* (2013) and Reeves and Wang (2015) to minimise sampling days and therefore required resources, whilst maintaining accuracy in measured N₂O emissions, compared to constant intensive sampling schedules. In the case of rainfall-based samplings in this study, the +Biweekly samplings for one week after rainfall resulted in on average the smallest increase in sampling days of 88% and 29% over the fixed Fortnightly and Weekly

schedules, respectively. Although the number of sampling days increased compared to their respective fixed sampling schedules, the rainfall-based sampling schedules still had 24% to 74% less sampling days than the Triweekly schedule.

The number of sampling days in event-based sampling schedules is determined by the frequency of emission-promoting events and can therefore change between years and sites (Table 1). In these relatively high rainfall sugarcane systems, the increased sampling after rainfall events effectively caused long periods of tri- or biweekly sampling. This was particularly true for Site 2, where there were 49 rainfall events > 20mm, especially when sampling was increased for two weeks following rainfall. This resulted in more samplings compared to the same sampling schedule at the other sites. By reducing the timeframe of increased sampling in a Weekly schedule from two to one week, sampling days were reduced between 8 and 33%, with minimal loss in accuracy.

Barton *et al.* (2015) recommended that global estimates of N₂O emissions from soils be revised using at least daily measurements or measurements taking into account the temporal variability of emissions. Our data demonstrate that the latter option can produce a satisfactory estimate of annual cumulative N₂O emissions from sugarcane soils. As a result of taking into account the accuracy, the variation between different sampling days and the number of sampling days, Weekly sampling with Biweekly sampling for one week after > 20 mm rainfall was the most efficient sampling schedule. This schedule resulted in on average a 29% increase in sampling days compared to the fixed Weekly schedule, but decreased the outcome variation and eliminated very high deviations (>20%), achieving a lower level of risk and consistently accurate estimation of annual cumulative N₂O emissions with an acceptable number of sampling days. As the selection of a sampling schedule needs to take into account both accuracy and practicality, it is acknowledged that some flexibility would be desirable in the implementation of a gas sampling schedule. For instance, in cases when rainfall is no longer a key driving factor of N₂O emissions, such as during the late cropping season after exhaustion of soil mineral N due to leaching, denitrification or plant uptake, a reduction in the sampling frequency following rainfall can be considered. Likewise, sampling frequency can also be reduced from Weekly to Fortnightly before rainfall if the N₂O emissions remain low due to restrictive site or seasonal conditions.

4.4. Spatial versus temporal variability and implications for measurement methods

Spatial variability could be a greater source of errors compared to the temporal variability as evidenced by the large differences in annual cumulative N₂O emissions measured from different plots (Fig. 5). It should be noted that due to the limited number of replicates at each site, the current dataset can only offer an indication of spatial variability, and the true spatial variability may be much smaller or larger. Therefore direct comparisons between temporal and spatial variability, in this case, does not provide a robust assessment of schedule suitability. However, the large spatial variability does not warrant ignorance of the potential errors caused from inadequate gas sampling frequency, rather it highlights the need to develop spatially representative measurement techniques. The large gas sampling chambers used in the present study would have helped reduce the impacts of spatial variability. However, more replicates are highly desirable in order to obtain representative measurements across a site. Although automatic gas sampling systems can produce temporally high resolution measurements, they can carry only relatively small numbers of chambers (mostly ≤12). Therefore, the capacity of an automatic gas sampling system is usually inadequate for comparing multiple different treatments in sugarcane fields, where the crops are often grown with wide row spacing on raised beds and fertiliser applied in bands. In this sense, the manual gas sampling method, which allows the use of a large number of chambers, will remain a useful tool for the measurement of N₂O emissions from sugarcane fields as long as adequate sampling frequencies are maintained as discussed above.

5. Conclusion

This study has provided the first assessment on sampling time of day and sampling frequency over a year in very high emission systems, characterised by high N inputs, high rainfall and high temperatures over summer. At the three sugarcane sites assessed, temperature-driven diurnal variation in emissions was evident. The time period with emissions closest to the daily mean occurred shortly before midnight (21:00 to 24:00) and late morning (09:00 and 12:00). Considering operational convenience, the period between 09:00 and 12:00 was the most appropriate sampling time of day.

Using data measured between 09:00-12:00 to assess sampling schedule effectiveness, instead of the daily mean emissions which is commonly used, resulted in similar or larger variation and error in the sampling outcomes. However, this is a more realistic representation of actual manual sampling in the field, and so should be considered for future sampling schedule assessments.

Rainfall was the major factor regulating day to day variation in N₂O emissions at all three sugarcane sites, particularly during the first 4-5 months after N fertiliser application. Because of the pronounced and prolonged high emission period over spring and summer, the annual cumulative N₂O emissions were less dominated by the highest emission peaks in sugarcane cropping compared to lower emission systems. Therefore, intermittent sampling schedules can achieve greater accuracy in higher emission systems compared to lower emission environments. Consequently, to attain a given accuracy fewer samplings are required in a higher emission system. The most efficient gas sampling schedule was Weekly sampling with Biweekly sampling for one week after >20mm rainfall due to its lack of extreme (>20%) deviations from the 'actuals', high probability of estimating the annual cumulative emissions within an acceptable precision and substantially reduced sampling numbers in comparison to fixed Triweekly or Biweekly samplings. Based on this recommended sampling schedule, appropriate adjustment of the sampling frequency can be made by the operators at very high or low emission times depending on expected accuracy and resource availability.

While addressing the temporal variability in N₂O emissions by means of an appropriate sampling schedule, is vital for obtaining a reliable measurement of whole season cumulative emissions, the use of a sufficient number of replicates is also paramount to minimise the impact of spatial variability. Therefore, manual gas sampling chambers, ideally those covering a relatively large area, will continue to be a useful tool for measuring N₂O emissions due to low setup costs and thus the potentially large number available for deployment. Further research is recommended to quantify the spatial variability of emissions within sugarcane cropping to elucidate which source of variability is limiting measurement accuracy and how temporal and spatial variability can be effectively addressed simultaneously.

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