Report on negative N_2O fluxes.

MAF Sustainable Land Management and Climate Change.

SLMACC Agreement LINX-1002

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Executive Summary

- This report is presented in 6 sections.

- In Sections 1 and 2 the gas chromatography technique used to generate N_2O datasets held by AgResearch and Landcare is assessed to determine the true detection limits for the analysis of N_2O in gas samples. These ranged from 11.3 ppbv to 16.6 ppbv. Then the data sets are interrogated and the detection limits applied, in order to determine the frequency of negative N_2O fluxes.

- AgResearch assessed 22 trials (11941 N2O flux determinations) finding 65% of fluxes were positive, 28% were within detection limits and just 7% were negative. Landcare examined 9 trials (8000 flux determinations) finding only 8 negative fluxes.

- A meta-analysis of the AgResearch data showed negative fluxes were favoured by low soil nitrate concentrations, increasing soil temperatures, and extremes of soil moisture.

- This same meta-analysis showed dung influenced the proportion of Negative fluxes.

- Section 3 reports on an inter-laboratory comparison of laboratories at Landcare and Lincoln. Gas standards were sent from each laboratory to the other and analysed 'blind'. The results show good agreement with no influence of laboratory on N_2O analyses when Lincoln standards were analysed. However, analysis of the Landcare standards did depend on laboratory for some of the standards analysed. Reasons for this must be clarified with a future round of comparisons but requires other laboratories to be involved.

- In Section 4 an experiment to monitor soil profile N_2O concentrations from 0 – 45 cm depth is described, and this is ongoing. But to date there have been no indications that the site selected (extensively managed low N inputs) has the potential to be an N_2O sink. All concentrations measured have exceeded ambient N_2O concentrations.

- An experiment was conducted to assess a method for determining negative N_2O fluxes (Section 5). Nitrous oxide labelled with the stable isotope ^{15}N was injected into the headspace along with a conservative gas tracer (SF_6). Decreases in N_2O-^{15}N enrichment indicated production of N_2O in situ while decreasing concentrations of SF^6 indicated physical diffusion of SF_6 into the soil. The net result was a positive N_2O flux. The method has the potential to be specifically applied to resolve N_2O flux mechanisms.

- Section 6 presents a summary of the preceding questions and notes that negative N_2O fluxes have indeed been observed in New Zealand's agricultural system. However, their low frequency and magnitude suggest they are not significant in off-setting positive fluxes. Suggestions for further work are suggested. In particular examining the role of dung in instigating negative fluxes

Section 1

Significance of negative N_2O fluxes in New Zealand pasture soils: Agresearch data analysis

Subcontract to Lincoln University

T.J. van der Weerden and F.M. Kelliher

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1. Summary

Agresearch was subcontracted to Lincoln University to assess the potential for pasture soils to act as N_2O sinks as part of a Ministry of Agriculture and Forestry- Sustainable Land Management and Climate Change (SLAMCC) funded project. The project involved:

- Determine detection limits of GCs used for N_2O work, and associated 95% confidence interval
- Determine the minimum detection limit of field-based N_2O flux measurements
- Collate MAF-funded Agresearch-conducted N_2O field trial data (urine, dung and control treatments) to assess the occurrence of 'true' negative fluxes (denoted as those below the lower boundary of the detection limit)
- Conduct a meta-analysis of all data to determine the influence of trial variables (treatments, location, soil variables) on the occurrence of negative fluxes
- Contribute to the discussion in the final report by summarising the conditions resulting in true negative fluxes

Analysis of the Agresearch GC and N_2O dataset reveals the following finding:

- The GC precision for the AgResearch GC4 was calculated to be 11.3 ppbv N_2O.
- The associated detection limit (DL) for the AgResearch data ranged from ±0.00256 to ±0.00664 mg N_2O-N/m^2/hr, averaging ± 0.00398 mg N_2O-N/m^2/hr, with variation due to differences in chamber design or sampling times.
- There was a grand total of 11941 N_2O flux measurements made from 22 trials across three regions (Waikato, Southern Hawkes Bay and Otago) conducted from 2000 to 2009,
- Of these, 3357 values (28%) lay within the DL,
- Positive fluxes comprised 65% of the data, while 7% were negative fluxes.
- Of the 22 trials, 2 trials conducted in the Southern Hawkes Bay resulted in an exceptionally large frequency of negative fluxes (31 and 38%). Excluding these data, the percentage of negative fluxes decline from 7% to 4%.
- Across all trials, the Southern Hawkes Bay had the highest frequency of negative fluxes (18% compared to 4-5% for Waikato and Otago). The reason for this observation is currently unknown.

- Excreta type influenced the frequency and magnitude of negative fluxes, with 13% of fluxes from dung being negative compared to 6% and 3% for control and urine treatments. Dung also has the lowest median flux value.
- Analysis of control plot data reveals no or very little change in frequency of negative fluxes over a 10 year period.
- Negative fluxes appear to be favoured by low soil nitrate-N content.
- Volumetric water content influences the frequency of negative fluxes, with a greater frequency occurring under very dry or very wet conditions. However, soil drainage class had no effect.
- Increasing soil temperature increased the frequency of negative fluxes.
- The frequency of calculated negative fluxes was slightly higher when based on 3 headspace samples compared to 2 collected from chambers, suggesting fluxes based on 2 headspace samples does not increase the occurrence of negative fluxes.

2. Detection limit of Agresearch gas chromatographs (GCs)

A detection limit (DL) quantifies the smallest value of N_2O emissions that can be reliably measured. The three variables affecting the DL are height of the soil cover (chamber) head space, the interval between gas samples taken from the headspace and precision of the GC N_2O concentration measurement system. The precision of a GC will quantify the variability of measurements for a set of samples, each having the same N_2O concentration. The smaller the variability the greater is the signal to noise ratio. This means a more precise GC and chamber system will be more able to detect truly negative N_2O emissions.

The Agresearch GC is located in a controlled temperature laboratory at Lincoln University. During 19 January 2011, we determined the precision of the GC (called GC4). This particular GC was used for determining precision as most of the air samples had been analysed by this instrument. There were four sets of 20 samples with N_2O concentrations of 200, 321.5, 500 and 1000 ppbv as well as a fifth set of 20 air samples obtained from a field trial site at Lincoln. The fifth set emulated the background air samples collected during N_2O emissions measurement field trials. With the five sets of samples, we investigated whether or not the precision has been affected by the N_2O concentration. This was based on a premise that precision of the GC's electron capture detector might be affected by the density of N_2O molecules in a sample. The inclusion of a sub-ambient concentration, 200 ppbv (ambient = 321.5 ppbv, see explanation below) was based on the principles of gas diffusion whereby a negative N_2O flux requires a sub-ambient concentration in the soil's pore space. We included three standards (200, 500 and 1000 ppbv) comprised of N_2O and nitrogen (synthetic air) as well as 'real' air (321.5 ppbv) in order to investigate whether or not the precision of synthetic air samples would be different to that of 'real' air. The GC is calibrated using a number of synthetic air standards as well as 'real' air from Greta Point, while of course, the samples are real air.

The three alpha standards had been purchased from BOC on 14 July 2010, having been mixed in Sydney, Australia, and analysed in Auckland to confirm the guaranteed 95% confidence interval (± 10 ppbv N_2O). The 'real' air came from a bottle that had been filled with air on 18 November 2009 outside NIWA's laboratory

at Greta Point, Wellington. The N_2O concentration of air contained in this bottle was measured by NIWA using another GC. NIWA's GC had been "calibrated" by standards certified by the United States National Institute for Standards Technology with 're-examination' of these standards done at Boulder, Colorado, during November 2010. An arithmetic mean N_2O concentration for 10 samples of the bottled air from Greta Point was 321.5 ppbv ± 0.1 ppbv (Gordon Brailsford, pers. comm.).

The five sets of 20 samples were analysed by the GC as well as fifteen check samples containing real air collected outside the laboratory and ten calibration standard samples. The total of 125 samples had been placed randomly in the auto-sampler racks. Each sample took 8 minutes, so 125 samples were analysed over a period of 16.7 ≈ 17 hours. Precision has been quantified by analyses of the sample peak areas. Thus, the GC calibration was not involved. The measure of precision was a standard deviation. A coefficient of variation (CV, %) can be computed by the standard deviation expressed as a percentage of the mean.

Results showed that the precision was virtually identical (CV = 1.70%) for synthetic air containing 200 and 500 ppbv N_2O, but 21% larger (CV = 2.06%) for synthetic air containing 1000 ppbv N_2O, an unexpected result opposite to an anticipated one. Overall, we concluded **the precision was not consistently nor significantly affected by the N_2O concentration.** While the samples used for this determination were either synthetic air samples or real air samples, additional analysis indicated that GC precision was not influenced by the origin of the sample. Likewise, a comparison of CVs for 'real' air from Greta Point sampled from the 321.5 ppbv bottle (CV = 0.1.07%) and 'real' background air sampled at a trial site (CV = 1.25%) suggested 'real' air samples from a trial site varied about the same as 'real' air samples from a bottle. To quantify GC precision for the meta-analysis, we used the CV of 1.25% for background air sampled at a trial site. For these samples, the arithmetic mean N_2O concentration was set to 321.5 ppbv, so the corresponding standard deviation would be 4.0 ppbv.

We have compared the GC precision, expressed as a percentage of the measured value, to that of overseas laboratories based on reports in articles published in peer-

reviewed journals. For a USDA laboratory at Fort Collins, Colorado, ten measurements of air with a mean N_2O concentration of 316 ppbv had a standard deviation of 1.0 ppbv, so their GC measurement system had a precision of 0.32% (Mosier and Mack 1980). Similarly, for the University of Edinburgh laboratory, twenty measurements of air with a mean N_2O concentration of 316 ppbv had a standard deviation of 1.32 ppbv, so their GC measurement system had a precision of 0.42% (Arah et al. 1994). Finally, for a CSIRO laboratory at Aspendale, Melbourne, Australia, the GC measurement system precision of replicate analyses was reported to be 0.3%, and while the statistic was not specified, it was assumed this was a standard deviation (Galbally et al. 2010).

The GC precision at Lincoln was different to that of three overseas laboratories. We wondered if there might be something different about the GC at Lincoln. One different feature of the GC system at Lincoln is the sample volume is determined by the volume of the sample container, called a vial. To explain, during a field or laboratory trial, a chamber headspace air sample is collected and stored in a 6 ml vial for GC analysis using a syringe. A 12 ml sample is injected into the vial (so the sample would be stored at twice atmospheric pressure), so there can be no contamination of the sample by incursion of (ambient) air. Prior to GC analysis, the air sample is returned to atmospheric pressure by inserting a (hollow) needle connected to a water-filled vessel (bubbling or bubbles indicating the reduction in sample pressure, bubbling stops when atmospheric pressure is reached). Thus, the volume of the air sample to be analysed by the GC is intended to be 6 ml, the vial volume. As a component of the GC precision, volume variability from one vial to another corresponds with variability in the air sample's volume. A GC detector responds to the N_2O molecules. Air includes N_2O, so for a given N_2O concentration, a sample of greater volume will include more N_2O molecules than a sample of lesser volume.

To examine the variability of sample volume, we measured the volume of 60 vials, 20 from each laboratory conducting trials in the Waikato, Ballantrae and Invermay sites. The volume was measured by an Archimedes method. A vial was carefully filled with water, judged by to a meniscus that formed at the top (opening) of the vial, and weighed inside a closed, glass cabinet on a sensitive (1 milligram

resolution) balance. To estimate the reliability of this method, the volume of a vial was repeatedly measured nine times yielding a standard deviation of 0.27% (CV = 0.27%). Measuring the volumes of 20 vials used for sampling at the Ballantrae site yielded a CV of 0.57%. To further check the method, a different person measured the volumes of 14 and 9 vials from the Waikato and Invermay and the corresponding CVs were 0.75 and 0.69%. Thus, broadly similar results (CVs) were obtained by two people measuring the volumes of three sets of vials. On average, the CV was 0.67%. This included a method (repeatability) error quantified by the CV of 0.27%. To eliminate the method error, a root mean square calculation was done, yielding the variability of sample volume quantified by a CV of 0.61% (= $\{[0.67^2] - [0.27^2]\}^{0.5}$).

The GC precision or variability of GC measurements of 'real' air samples from a trial site was quantified by a CV = 1.25% for GC4. One component of this GC precision may be attributed to the variability of air sample volume. Measurements indicated this component of the GC precision was quantified by a CV of 0.61%. If the GC precision had a CV of 1.25% and the variability of air sample volume a CV of 0.61%, we deduced that 51% of the GC precision ([1 – {0.61%/1.25%} = 0.51) or variability of GC measurements of air samples could be attributed to the GC's electron capture detector.

3. Determination and frequency of negative fluxes

3.1 Data compilation

Data from 22 MAF-funded Agresearch field trials conducted from 2000 to 2009 were compiled in MS Excel. Data was collated at the replicate level, where treatments were limited to animal excreta (dung and urine) and control (no N source applied). Independent variables such as soil temperature, soil water content and soil mineral N content was also collated to allow for a metadata analysis of the dataset.

In total, there were 11941 N_2O flux measurements collected from 10 different pastoral soils in three key regions (Waikato, Southern Hawkes Bay and Otago) across two soil drainage classes (well and poorly drained).

All N_2O flux data is presented in the units of mg N_2O-N/m^2/hr. To provide some context to the data, fluxes from a urine patch will typically range from 0 to 5 mg N_2O-N/m^2/hr.

3.2 Gas sampling method

Static chamber design

For 21 of the 22 trials a static chamber with a rim on the lower edge was inserted into the soil. The length of the rim varied from site to site. In the Waikato, chambers were inserted 50-100mm into the soil, following the method employed by Luo *et al.* (2008), while in the Southern Hawkes Bay chambers were inserted ~20 mm (C. Hoogendoorn, pers. comm.; 7 February, 2011). Following discussions with Dr. Rob Sherlock, Lincoln University, the static chamber design used in Otago evolved from a single chamber with a rim inserted ~30 mm (de Klein *et al.* 2003), employed in all but one of the Otago MAF-funded N_2O trials, to a separate chamber base and top. The base incorporated a water-filled trough, which, when filled, would ensure a gas-tight system when the top was positioned into the base. The rim on the base was lengthened to allow it to be inserted ~90 mm into the soil. This new design was employed in the latest of the 22 MAF-funded trials (Otago hill country trial in 2009), and has since been used in all N_2O research in Otago.

Gas sampling procedure

Following treatment application, gas samples were collected twice per week for the first month and then once per week or fortnightly until background levels were reached. Additional gas samples were collected following significant (e.g. > 10 mm) rainfall events to ensure the influence of this key driver on N_2O production and emission was captured. On each sampling day, static chambers would be positioned over the treated plots and N_2O measurements were carried out between 12 noon and 2 p.m.

In 10 of the 22 trials, for the first month following treatment application, three headspace gas samples were taken during a cover period of 60 minutes at times 0 (t0), 30 (t30) and 60 minutes (t60) from each chamber using syringes and transferred into a 6 ml septum-sealed screw-capped glass vial. The method used for collecting headspace samples (Fig. 1) was developed in the first seasonal EF3 trial (de Klein et al., 2003). After one month, headspace sampling would be reduced to two samples, collected at t0 and t60. However, in 12 of the 22 trials, gas sampling was restricted to two headspace samples being collected from each chamber for the entire trial period, based on confidence in linearity of concentrations obtained from earlier trials. Across all 22 trials and 11941 individual flux measurements, 9805 (or 82%) were based on 2 headspace samples and 2136 (or 18%) were based on 3 headspace samples.

Gas sample vials were over-pressurized to ensure sample integrity was maintained: each vial was tested in the laboratory by inserting a double-ended needle, connected to a tube positioned in a beaker of water, into the vial immediately prior to gas analysis. Production of air bubbles in the beaker demonstrated that a positive pressure had been

maintained and thus no leakage occurred. Sampling was discontinued when N_2O fluxes from excreta treatments had returned to the levels measured from control plots.

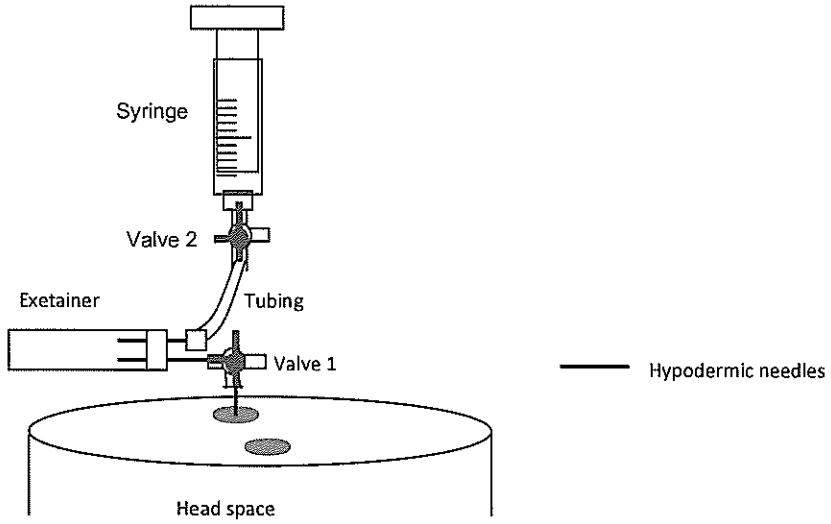

Figure 1: Schematic diagram of the headspace sampling device (Source: de Klein et al., 2003)

3.3 Determining true negative fluxes

Before negative flux data could be collated into a single dataset, it was necessary to exclude flux data that lay within the detection limit (DL).

To quantify the DL for meta-analysis, we begin by writing an equation for the N_2O flux measured by the chamber method as:

$$F_{N2O} = (\delta c/ \delta t)*(M/V_m)*(V/A) \quad (1)$$

where δc is change of N_2O concentration in the chamber headspace during an enclosure period (μl/L), δt the enclosure period (h), M the molar weight of N in N_2O (g/mol), V_m the molar volume of gas at the sampling temperature (l/mol), V the headspace volume (m^3) and A the area covered (m^2); the headspace height is (V/A). For analysis, we can write a simplified flux equation as:

$$F_{N2O} = U\ (c_2 - c_1) \quad (2)$$

where c_2 and c_1 are the N_2O concentrations at the end and beginning of an enclosure period, and U will subsume the other (constant) terms in eqn (1). We can assume c_2 and c_1 are independent, so the variance of F_{N2O} ($Var[F_{N2O}]$) can be written:

$$Var[F_{N2O}] = U^2*(Var[c_2])^2 + Var[c_1])^2) \quad (3)$$

A standard deviation (SD) is the square root of the variance and by writing c_a as the average of c_2 and c_1, eqn (3) may be re-written as

$$SD[F_{N2O}] = U*2^{0.5}*SD[c_a] \quad (4)$$

To illustrate use of the analysis, an example set of calculations will be done. Firstly, we require a value for term U. For δt = 0.33 h, [V/A] = 0.1 m, air temperature = 10°C at sampling, and units of F_{N2O} and c_a equal to mg N m^{-2} h^{-1} and ppmv, respectively, U will be 0.365. Through term U, we can visualise how the chamber method "detection" limit will be affected by δt and [V/A]. If we increase (only) δt, *U* will decrease. For example, for δt of 0.50 and 0.67 h, U becomes 0.24 and 0.18, respectively. In contrast, if we increase (only) [V/A], U will increase. For [V/A] of 0.15 and 0.20 m, U becomes 0.55 and 0.73, respectively.

As has been shown, the DL is a function of three independent variables. Firstly, precision data from GC analyses (as described in Section 2) indicated $SD[c_a]$ was 4.0 ppbv. As shown by eqn (4), this value needs to be multiplied by ($2^{0.05}$) and then doubled for 95% confidence, yielding 11.3 ppbv (= $SD[c_a] * 2^{0.05} * 2$). This precision of 11.3 ppbv will be assumed to represent all GC's used for analysis of AgResearch gas samples. The other two independent variables that can affect the DL will be height of the soil cover used to measure N_2O fluxes (headspace height of the static chamber) and the interval between gas samples taken from the headspace to determine N_2O fluxes. The DL will increase with increasing headspace height, and decrease with increasing precision and sampling interval (Figure 2). The three variables sometimes changed from one trial to another, so DL ranged from ±0.00256 to ±0.00664 mg N_2O-N/m^2/hr. While a DL was determined for each trial, an average MDL was ± 0.00398 mg N_2O-N/m^2/hr (n = 11941).

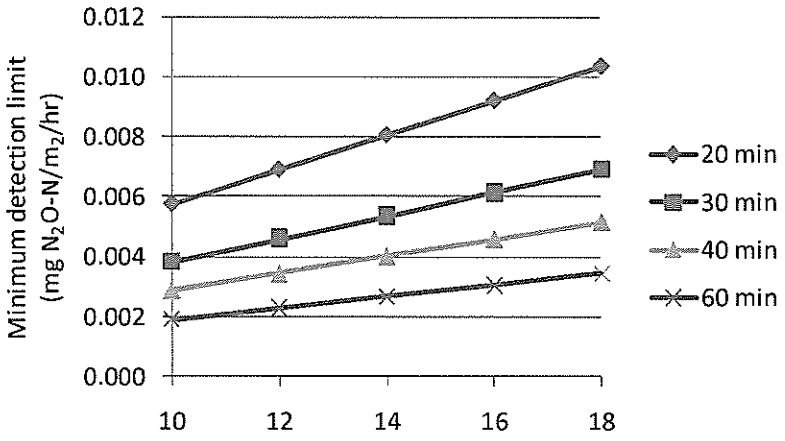

Figure 2: Relationship between the detection limit (DL) of N_2O fluxes and soil cover headspace height for different gas sampling intervals. The calculation of DL included the GC precision (95% confidence limit of 11.3 ppbv N_2O).

The flux data was disaggregated into three classes. Values greater than the upper boundary of the DL, exceeding twice the standard deviation, were denoted positive N_2O fluxes. Values within the DL bounds, twice the standard deviation, were considered equal to zero. Finally, values less than the lower boundary of the DL were denoted negative N_2O fluxes.

There were a grand total of 11941 N_2O flux measurements with 3357 values (28%) within the DL. Negative fluxes comprised 7% of the data, with values ranging from the smallest DL of -0.0019 to -0.1056 mg N_2O-N/m^2/hr. Positive fluxes comprised 65% of the data. Interestingly, the frequency of positive flux values lying between the absolute range of negative values (i.e. 0.0019 to 0.1056 mg N_2O-N/m^2/hr) was approximately half, with 6565 of the 11941 flux measurements, or 55%, being positive fluxes within this range.

During trials 16 and 17, for unknown reasons, the frequency of negative fluxes was exceptionally large, 31 and 38%, respectively. Excluding the data from these trials, there would be a grand total of 10892 measurements with 29% within the DL, while 67% and 4% of the values were positive and negative fluxes, respectively.

An analysis of the entire flux dataset and the negative flux dataset shows that the median flux was 0.0076 mg N_2O-N/m^2/hr and -0.0068 mg N_2O-N/m^2/hr, respectively.

A very large proportion of the negative fluxes were very close to zero, with 70% of the values lying between the lower DL boundary of -0.0019 and 0.01 mg N_2O-N/m^2/hr (Fig. 3).

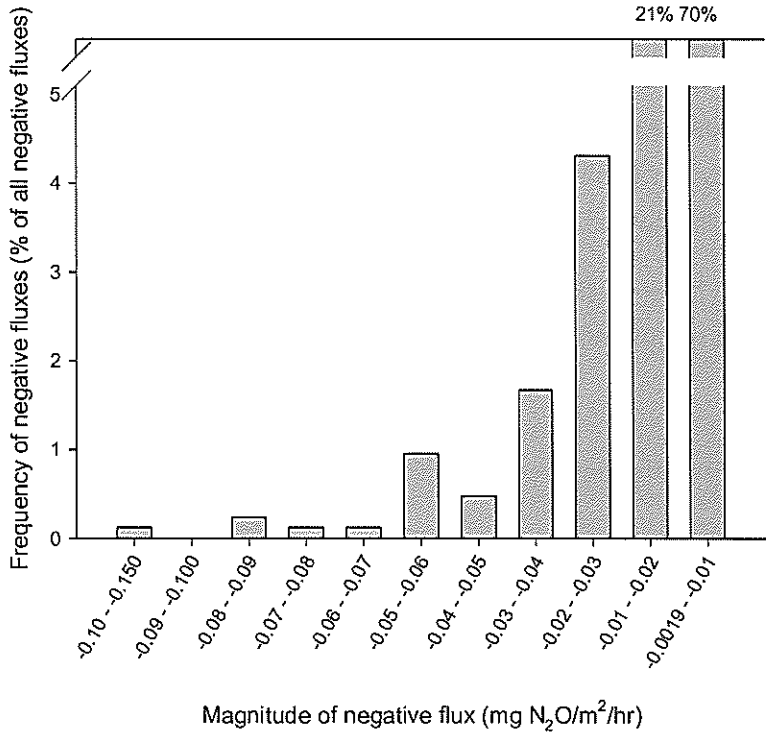

Figure 3: Frequency of negative fluxes.

Negative fluxes were detected in all but one of the 22 MAF-funded N_2O trials conducted by Agresearch between 2000 and 2009 (Table 3). All trials had a common objective: to determine the EF3 value for excreta treatments applied to pastoral soils under contrasting soil and climatic conditions: details on the conditions for each trial are provided within Table 3.

The single trial that had no negative fluxes detected was the very first trial, conducted in the autumn of 2000. The method for calculating fluxes in this first trial was similar to that used in the remaining 3 seasonal EF3 trials conducted from 2002 to 2003. The only difference between this trial and others is the soil type (poorly-drained Warepa silt loam). This soil was not used in subsequent trials due to a change in trial location. However, the emissions factors calculated from this first trial are similar to those calculated in subsequent trials conducted on the poorly drained Otokia silt loam in Otago.

At the trial level, negative fluxes, as a percentage of all data, ranged from 0% to 38%, with the largest proportion (31-38%) being associated with trials determining EF3 values from sheep urine on hill country at Ballantrae under moderately low stocking rates in 2009. When these two trials are excluded, negative fluxes as a percentage of all data decreased from 7% to 4%.

To examine changes in occurrence of negative fluxes over time, data was limited to control plots for the three regions (Fig. 4). The results for Otago suggest there is no change in the frequency of negative fluxes over time, with Waikato results showing a

similar level of frequency. The GC precision was assessed on GC4, which was purchased in 2008. Samples collected from trials before this date (i.e. 2000-2003) were analysed on a different GC at Lincoln University. Unfortunately we are not able to assess the precision of those earlier machines as they are no longer in use. However, no change in the frequency of negative fluxes over time from Otago would suggest the GC precision associated with the earlier GC(s) is similar to that for GC4.

Interestingly, the Southern Hawkes Bay region produced a larger frequency of negative fluxes: this is further discussed in Section 4.3.

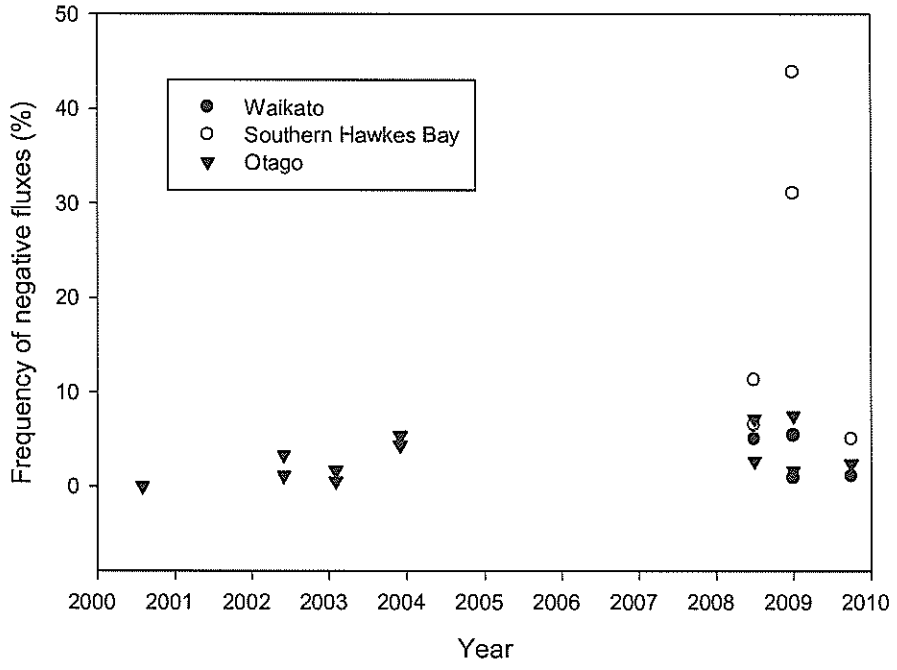

Figure 4: Frequency of negative fluxes over time measured from control treatments for the three regions used for AgResearch N_2O trials.

Table 3. Negative fluxe occurrence within individual MAF-funded AgResearch N_2O trials, including detail on trial treatments and conditions.

Trial No	Period of study	Season	Landuse	Region	Location	Soil type	Drainage class	Mean VWC (%)	Mean soil temp	Treatments[1]	No. of Reps	No. flux values	No. within MDL	No of neg. fluxes	% of fluxes within MDL	% of neg. fluxes
1	May-Oct 2000	Autumn	Lowland	Otago	Invermay	Warepa zl	Poor	50	7.4	C, DU, SU	4	752	31	0	4%	0%
2	Feb-Oct 2002	Summer	Lowland	Otago	Invermay	Otokia zl	Poor	52	10	C, DU	4	366	140	8	38%	2%
3	Feb-Oct 2002	Summer	Lowland	Otago	Invermay	Wingatui zl	Well	55	10	C, DU	4	365	50	2	14%	1%
4	Oct 02 - July 2003	Spring	Lowland	Otago	Invermay	Otokia zl	Poor	33	14	C, DU, SU, SD	4	918	508	12	55%	1%
5	Oct 02 - July 2003	Spring	Lowland	Otago	Invermay	Wingatui zl	Well	43	14	C, SU, SD	4	720	285	16	40%	2%
6	Aug 03 - Mar 04	Winter	Lowland	Otago	Invermay	Otokia zl	Poor	34	15	C, DU, SU, DD	4	719	308	20	43%	3%
7	Aug 03 - Mar 04	Winter	Lowland	Otago	Invermay	Wingatui zl	Well	45	13	C, DU, SU, DD	4	722	242	21	34%	3%
8	May-Sept 08	Autumn	Lowland	Waikato	Ruakura	Te Kowhai zl	Poor	49	10	C, DU, DD, SD	4	497	43	33	9%	7%
9	May-Sept 08	Autumn	Lowland	Waikato	Ruakura	Horotui zl	Well	49	10	C, DU, DD, SD	4	392	34	24	9%	6%
10	May-Sept 08	Autumn	Hill Country	Southern HB	Ballantrae	Ngamoko	Well	52	9	C, DU, BD, SD	4	492	96	32	17%	7%
11	May-Sept 08	Autumn	Hill Country	Southern HB	Ballantrae	Wilford	Poor	47	9	C, DU, BD, SD	4	495	69	49	17%	10%
12	May-Sept 08	Autumn	Lowland	Otago	Invermay	Otokia zl	Well	53	8	C, DU, DD, SD	4	559	130	65	25%	12%
13	May-Sept 08	Autumn	Lowland	Otago	Invermay	Wingatui zl	Poor	56	6	C, DU, DD, SD	4	448	69	10	11%	2%
14	Oct 08-Apr 09	Spring	Lowland	Waikato	Ruakura	Te Kowhai zl	Poor	32	20	C, DU, DD, SD	4	560	93	41	16%	7%
15	Oct 08-Apr 09	Spring	Lowland	Waikato	Ruakura	Horotui zl	Well	35	20	C, DU, DD, SD	4	447	31	13	5%	3%
16	Oct 08-Apr 09	Spring	Hill Country	Southern HB	Ballantrae	Ngamoko	Well	33	16	C, DU, BD, SD	4	526	141	163	49%	31%
17	Oct 08-Apr 09	Spring	Hill Country	Southern HB	Ballantrae	Wilford	Poor	32	18	C, DU, BD, SD	4	523	29	200	52%	38%
18	Oct 08-Apr 09	Spring	Lowland	Otago	Invermay	Otokia zl	Well	28	15	C, DU, DD, SD	4	600	190	74	30%	12%
19	Oct 08-Apr 09	Spring	Lowland	Otago	Invermay	Wingatui zl	Poor	32	15	C, DU, DD, SD	4	476	84	21	13%	4%
20	Sept - Dec 09	Spring	Hill Country	Waikato	Whatawhata	Dunmore	Well	57	16	C, DU, SU	5	448	135	4	11%	1%
21	Sept - Dec 09	Spring	Hill Country	Southern HB	Ballantrae	Wainui	Poor	54	13	C, DU, SU	5	499	361	20	58%	4%
22	Sept - Dec 09	Spring	Hill Country	Otago	Hindon	Kiteroa	Well	40	11	C, DU, SU	5	417	218	8	19%	2%

[1] C = control, DU = dairy urine, DD = dairy dung, BD = beef dung, SU = sheep urine, SD= sheep dung.

4. Meta-analysis of negative fluxes

A meta-analysis was conducted on all negative flux data, where the occurrence and associated median value was assessed on the basis of location and treatment (region and excreta type). Furthermore, data was analysed against soil variables (soil drainage class, volumetric water content, soil temperature and soil mineral N content).

4.1 Influence of treatments and location

Region

All Agresearch trials were conducted in one of three regions: Waikato, Southern Hawkes Bay and Otago. The majority of the data (59%) was collected from Otago, primarily due to the initial Agresearch-conducted seasonal EF3 studies from 2000 to 2003 being run in this region, with other key regions being managed by other research providers over this period (Lincoln University covering Canterbury and Landcare Research covering Waikato). The remaining data was equally distributed between Waikato (20%) and Southern Hawkes Bay (21%).

The Southern Hawkes Bay region resulted in the largest occurrence of negative fluxes, with 18% of all data being negative (Fig. 5). Waikato and Otago had similar proportions, at 5 and 4%, respectively. The median flux values also varied with region, with Waikato having the lowest (most negative) median value of -0.013 mg N_2O-N/m^2/hr while the median value for Otago was the highest at -0.005 mg N_2O-N/m^2/hr.

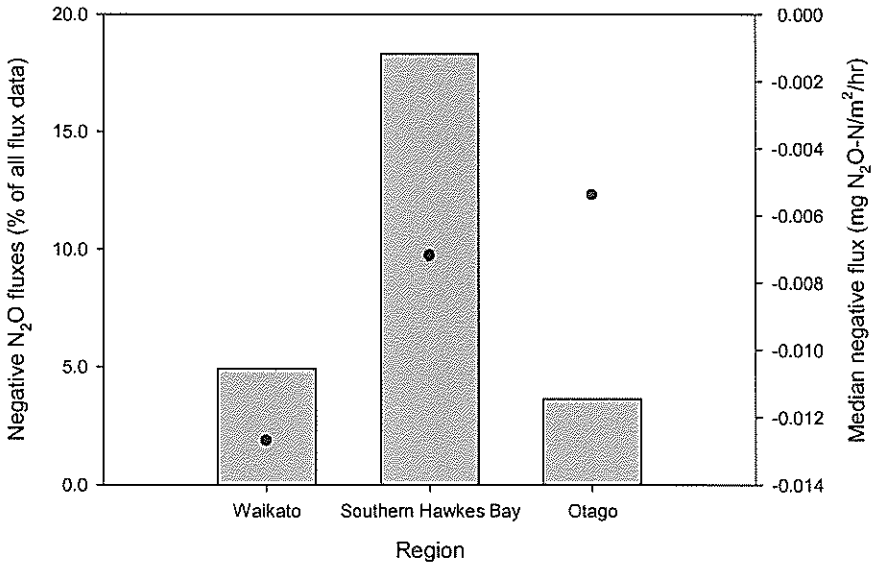

Figure 5: Influence of region on occurrence and magnitude (• = median value) of negative fluxes.

Excreta type

The proportion of flux measurements from the three excreta treatments assessed for this project (urine, dung and control) were 34%, 38% and 28% of all measurements, respectively.

Dung treatments resulted in the largest occurrence of negative fluxes (13% of all dung flux data) and the lowest median flux (-0.0071 mg N_2O-N/m^2/hr; Fig. 6). This result was strongly influenced by animal type, where the frequency of negative fluxes was greatest with beef dung (Fig. 7). Beef dung also had the lowest (most negative) median negative flux, at -0.0082 mg N_2O-N/m^2/hr. It is interesting to note that the beef dung trials were conducted in the Southern Hawkes Bay at Ballantrae, which influenced the large proportion of negative fluxes detected from this region (Fig. 4 and 5). Ballantrae is a hill country station, where pastures are grazed less intensively compared to lowland sites.

By pooling all flux data, negative fluxes account for 7% of all measurements; however, when restricting the dataset to the Ballantrae site, the proportion more than doubles to 18% of all measurements. This increased proportion of negative fluxes was found for all animal type x excreta type combinations, apart from beef dung, which was only measured at the Ballantrae site, and dairy dung, which has not been measured at Ballantrae (Fig. 7 *vs* Fig. 8). The Ballantrae site does not appear to influence the magnitude of negative fluxes, as the median negative flux values for control, dairy urine and sheep dung treatments are similar from the Ballantrae sites and all pooled data (Fig. 7 vs Fig. 8).

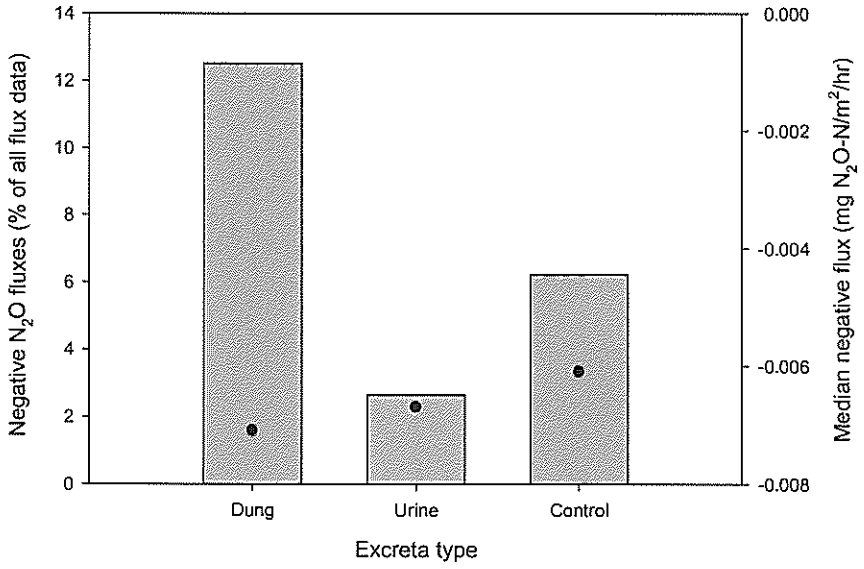

Figure 6: Influence of excreta type on occurrence and magnitude (• = median value) of negative fluxes.

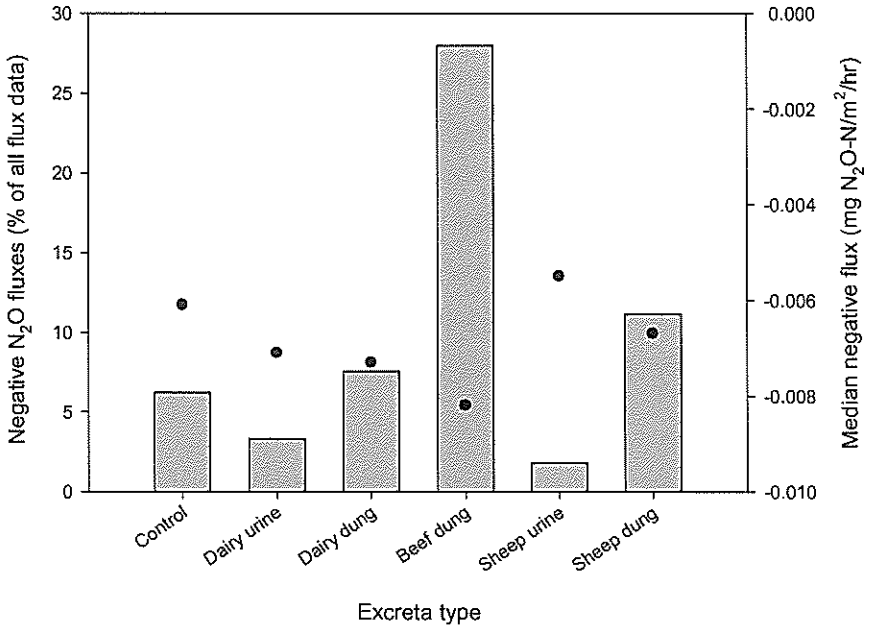

Figure 7: Influence of animal and excreta type on occurrence and magnitude (• = median value) of negative fluxes.

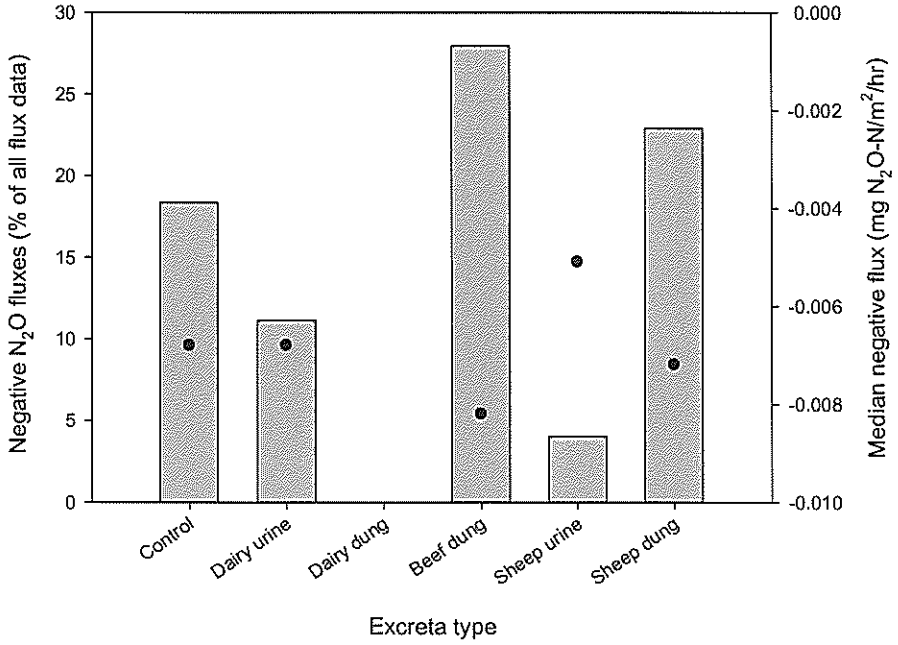

Figure 8: Influence of animal and excreta type on occurrence and magnitude (• = median value) of negative fluxes measured at Ballantrae hill country station in Southern Hawkes Bay (Note: dairy dung has not been assessed at Ballantrae).

4.2 Influence of soil variables

All flux data was pooled to examine the influence of soil variables on negative fluxes.

Soil mineral N content

Relating the occurrence of negative fluxes as a percentage of all data to the soil mineral N content suggests that soil ammonium-N content has little influence on the frequency of negative fluxes (Fig. 9). However, there appear to be more negative flux values measured when soil nitrate-N levels are low (Fig. 10). A similar finding has been observed by others, as reported by Chapuis-lardy *et al.* (2007). For nitrate, on average 8% of the measured fluxes were negative when soil nitrate-N content was less than 10 mg N/kg dry soil. This may be due to denitrifiers exhausting the supply of soil N_2O thus causing a diffusion of atmospheric N_2O into the soil. Of course, anaerobic conditions would be a requisite for such an occurrence. The median negative flux values ranged between -0.0053 and -0.0106 mg N_2O-N/m^2/hr for the soil ammonium data (Fig. 9), while the range was larger for soil nitrate, ranging from -0.0031 to -0.0312 mg N_2O-N/m^2/hr (Fig. 10) due to the very low median value calculated for data lying between 50 and 60 mg NO_3-N/kg dry soil. This value is based on only two data points, both being dairy urine treatments, thus less emphasis should be placed on this value.

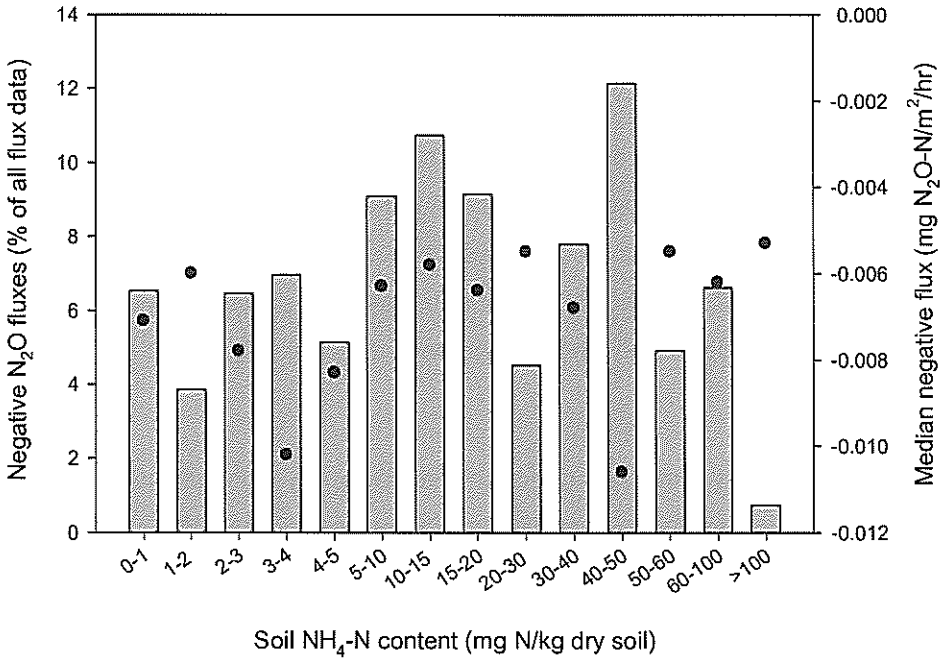

Figure 9: Influence of soil ammonium-N content (mg N/kg dry soil) on occurrence and magnitude (• = median value) of negative fluxes (note that the X-axis ranges are irregular).

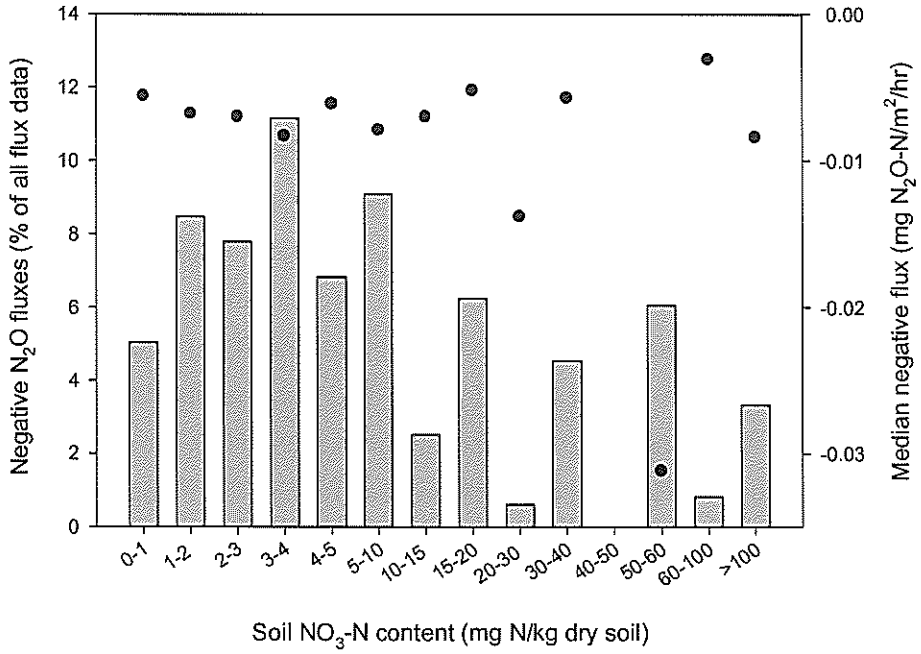

Figure 10: Influence of soil nitrate-N content (mg N/kg dry soil) on occurrence and magnitude (• = median value) of negative fluxes (note that the X-axis ranges are irregular and the right hand side y-axis scale differs from other figures).

Soil drainage class

Flux measurements were relatively evenly distributed across well drained (46%) and poorly drained soils (54%). Negative flux data represented approximately 7% of all flux data for each drainage class (Fig. 11), suggesting no influence of this variable on the extent of negative fluxes occurring. The median negative flux for each drainage class was also similar, at -0.0070 and -0.0063 mg N_2O-N/m^2/hr for poorly drained and well drained soils, respectively.

Volumetric water content

Volumetric water contents (v/v,%) has a marked effect on the frequency of negative fluxes (Fig. 12). The frequency was greatest under dry soil conditions, decreasing as the VWC increased to 50-60% v/v. However, the frequency of negative fluxes increased again as the VWC continued to increase up to above 60% v/v. The magnitude of the median fluxes was relatively similar, ranging from -0.0045 to -0.0079 mg N_2O-N/m^2/hr.

The trend observed between VWC and frequency of negative fluxes was investigated further by determining if a similar trend would be observed for positive fluxes. By limiting positive flux data to the equivalent but absolute range of negative fluxes (i.e. 0.0019 to 0.1056 mg N_2O-N/m^2/hr) (see Section 3.3), the frequency of positive fluxes versus VWC was plotted (Fig.12). This contrasting analysis suggested a similar but opposite pattern to the negative flux data, with the

lowest frequency of positive fluxes (20%) occurring under dry soil conditions (< 20% VWC), while the frequency was similar, averaging 54% of all data, when soil moisture was above 20% VWC.

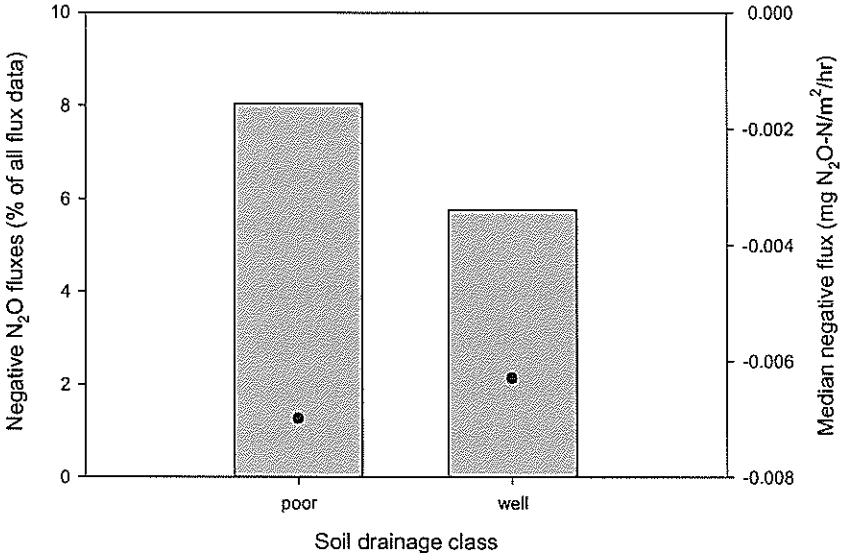

Figure 11: Influence of soil drainage class on occurrence and magnitude (• = median value) of negative fluxes.

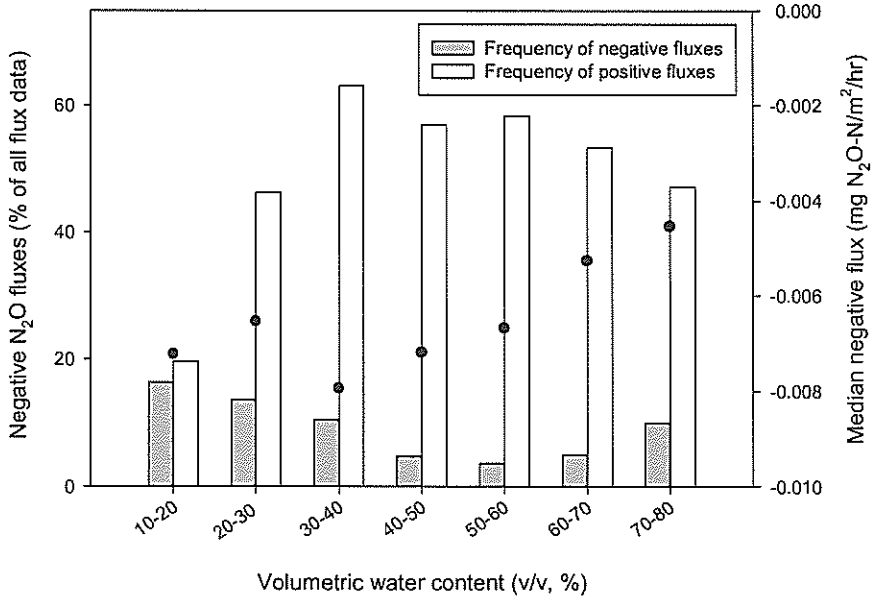

Figure 12: Influence of volumetric water content (v/v %) on frequency (grey bars) and magnitude (• = median value) of negative fluxes. Also shown is frequency of positive fluxes (white bars) over equivalent range of values (see text).

Soil temperature

Average daily soil temperature was measured at either 5 or 10 cm depth, depending on the trial. Data from both depths was pooled, as analysis of independent data suggests there is little difference when presented as the average daily temperature (data not shown).

The proportion of negative fluxes increased with increasing soil temperature (Fig. 13). The magnitude of the negative fluxes was similar for soil temperatures below 20°C, with median flux values ranging from -0.0056 to -0.0071 mg N_2O-N/m^2/hr. However, at soil temperatures between 20 and 25°C the median flux was markedly lower, at -0.0101 mg N_2O-N/m^2/hr.

As for VWC (Fig. 12), the trend observed between soil temperature and frequency of negative fluxes was investigated further by determining if a similar trend would be observed for positive fluxes. Positive flux data was limited to the equivalent but absolute range of negative fluxes (i.e. 0.0019 to 0.1056 mg N_2O-N/m^2/hr) to determine the frequency of positive fluxes versus soil temperature. This contrasting analysis suggested that soil temperature did not influence the frequency of positive fluxes, which ranged from 47 to 62% across the 5 soil temperature bands (Fig.13). We therefore conclude that soil temperature does have a significant effect on the frequency of negative fluxes, but not on the frequency of positive fluxes.

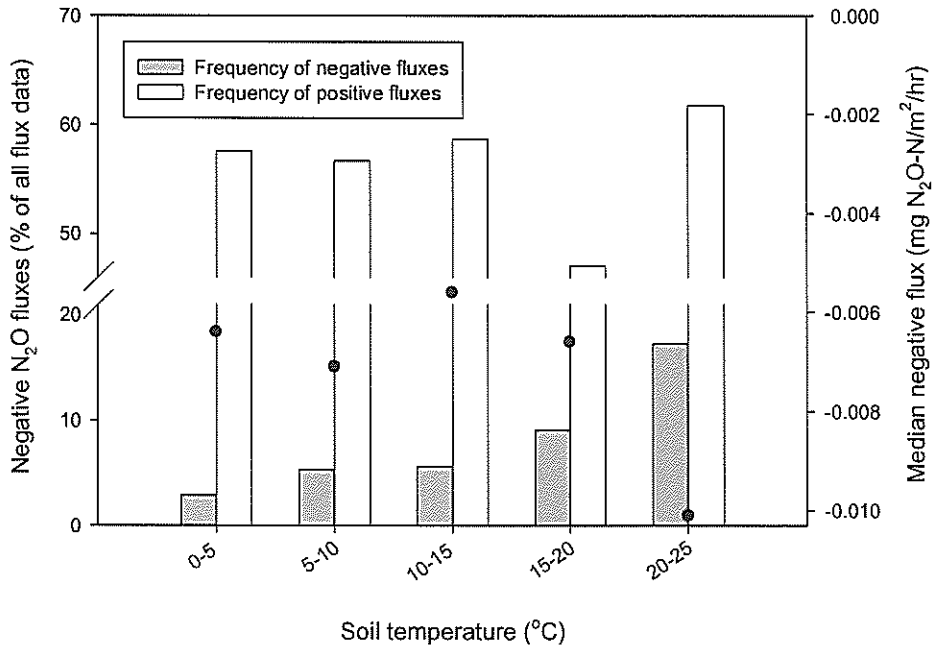

Figure 13: Influence of soil temperature on frequency of negative fluxes (grey bars) and magnitude of negative fluxes (• = median value). Also shown is frequency of positive fluxes (white bars) over the equivalent range of values (see text).

The reason for such a clear temperature effect is not fully understood. However, one possible explanation is increased carbon dioxide (CO_2) respiration with increasing soil temperature. A

high CO_2 concentration in the gas sample vials may cause interference with the GC determination of N_2O concentrations (Dr. Rob Sherlock, pers.comm.), which may lead to perceived negative fluxes when in reality the fluxes were very low or zero. This proposition will require further investigation by our colleagues at Lincoln University.

It is interesting that the frequency of negative fluxes generally decreases with increasing VWC but increases with increasing soil temperature. This observation is influenced by the general negative relationship between VWC and soil temperature, which is plotted using soil data limited to the negative flux dataset (Fig. 14). During cool winter months, soils are typically wetter due to lower ET and plant growth, whereas during warmer summer months pasture production and removal is greater while ET is also greater. A similar relationship was observed (not shown) when using soil data collected from all trials ($y = -0.237x + 23.78$; $R^2 = 0.53$; $P < 0.001$).

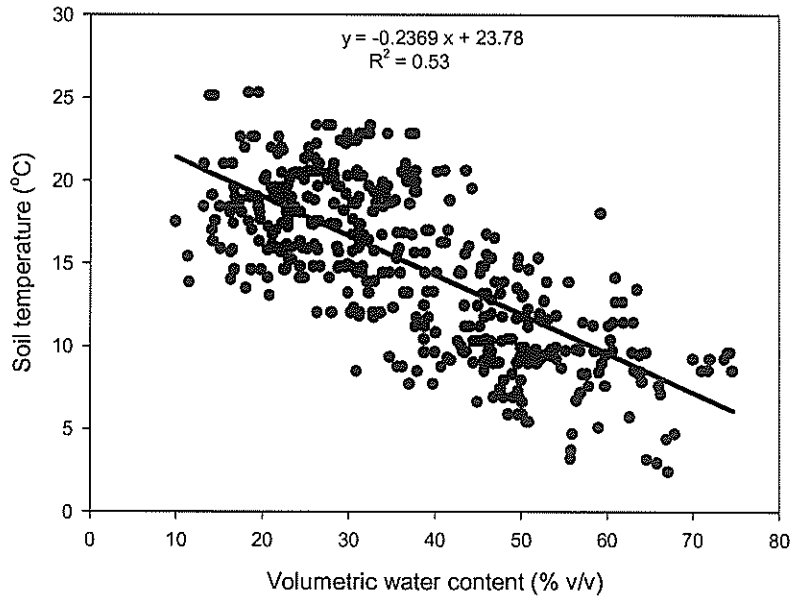

Figure 14: Relationship between volumetric water content and soil temperature. Soil data limited to occasions when negative fluxes were detected.

4.3 Southern Hawkes Bay data.

The analysis from above suggests that the Southern Hawkes Bay region results in a large frequency of negative fluxes (Fig. 4 and 5) across all treatments apart from sheep urine (Fig. 8). It is unclear why emissions measured at the Ballantrae hill country station have a greater frequency of negative fluxes compared to other sites: many variables may be contributing to this result. Trials 16 and 17, conducted at Ballantrae, resulted in the highest frequency of negative fluxes, with 31 and 38% of all data being negative (Table 3). When examining the average soil conditions, these sites were warm (16 and 18°C). However, there were 2 other trials (in the

Waikato) with warmer average soil temperatures (20°C) but similar average VWC (mean VWC of 32.5% v/v and 33.5% v/v for Ballantrae and Waikato sites, respectively) where the frequency of negative fluxes at the Waikato sites were lower at 3 and 7% of all data.

While soil moisture levels were low during trials 16 and 17 at Ballantrae, averaging 32.5% v/v, there were three other trials with similar or lower soil moistures that led to a lower occurrence of negative fluxes (trials 14, 18 and 19: Table 3). While trials 18 and 19 were also influenced by cooler average soil temperatures, which may have lowered the frequency of negative fluxes, warmer soil temperatures were measured at trial 14.

It is therefore necessary to explore potential artefacts that may lead to a greater occurrence of negative fluxes. One such artefact is chamber design. As noted in section 3.2, the chambers employed at Ballantrae are inserted to a depth of ~20 mm. This is the most shallow depth of all the sites, although the earlier chamber design employed in Otago was inserted only a little deeper, to ~30 mm depth. Under dry soil conditions it is possible the headspace is inadequately sealed due to very small gaps between the chamber rim and the soil matrix. Of course this alone will not lead to net negative flux activity. However, under windy conditions a negative pressure may develop in the headspace due to the Venturi effect, forcing air to be drawn into the soil, which may reduce the concentration of N_2O being sampled concurrently. To explore this further, windspeed data was obtained for the Ballantrae Spring Dung trial (trials 16 and 17) and compared with the magnitude of negative fluxes measured from these trials. Analysis showed no relationship between windspeed and negative fluxes, eliminating the possibility of chamber leakage due to wind as a cause of the measured negative fluxes.

4.4 Influence of number of headspace samples

As part of the data analysis, the influence of 2 versus 3 headspace samples on the occurrence of negative fluxes was assessed. The current AgResearch practice for gas sampling requires 3 headspace samples to be collected for the first 6 weeks, thereafter this is reduced to 2 headspace sampling. To determine if the number of headspace samples influences the frequency of negative fluxes, data was limited to control treatments (no excreta applied) where the sampling interval was always 30 minutes. In total, 1610 flux measurements fitted into this category. These were separated into those determined using 2 and 3 headspace samples.

The data suggested a higher frequency of negative fluxes when calculated from 3 headspace samples (5.3%) compared to fluxes calculated from 2 headspace samples (2.6%; Table 4). It is not possible to determine if this difference was significant, however it does alleviate any potential concern regarding increased occurrence of calculated negatives fluxes when based on 2 headspace samples. On average, there was a 3.4% occurrence of negative fluxes from control plots based on a 30 minute sampling interval (Table 4).

Table 4: Influence of number of headspace samples collected on occurrence of negative fluxes. Data limited to control treatments where headspace samples were collected at 30 minute intervals.

No. of headspace samples	Total no. of N_2O flux values.	No. of flux values within MDL	No. of negative flux values	Frequency of negative fluxes (% of total)
2	1177	549	31	2.6%
3	433	193	23	5.3%
Total	1610	742	54	3.4%

5. Conclusions

An analysis of the AgResearch GC system and AgResearch N_2O datasets revealed the following information about the frequency of negative fluxes and their prevailing conditions:

- The GC precision for the AgResearch GC4 was calculated to be 11.3 ppbv N_2O.
- The associated detection limit (DL) for the AgResearch data ranged from ±0.00256 to ±0.00664 mg N_2O-N/m^2/hr, averaging ± 0.00398 mg N_2O-N/m^2/hr.
- There was a grand total of 11941 N_2O flux measurements made from 22 trials across three regions (Waikato, Southern Hawkes Bay and Otago) conducted from 2000 to 2009,
- Of these, 3357 values (28%) lay within the DL,
- Positive fluxes comprised 65% of the data, while 7% were negative fluxes.
- Of the 22 trials, 2 trials conducted in the Southern Hawkes Bay resulted in an exceptionally large frequency of negative fluxes (31 and 38%). Excluding these data, the percentage of negative fluxes decline from 7% to 4%.
- Across all trials, the Southern Hawkes Bay had the highest frequency of negative fluxes (18% compared to 4-5% for Waikato and Otago). The reason for this observation is currently unknown.
- Excreta type influenced the frequency and magnitude of negative fluxes, with 13% of fluxes from dung being negative compared to 6% and 3% for control and urine treatments. Dung also has the lowest median flux value.
- Analysis of control plot data reveals no or very little change in frequency of negative fluxes over a 10 year period.
- Negative fluxes appears to be favoured by low soil nitrate-N content.

- Volumetric water content influences the frequency of negative fluxes, with a greater frequency occurring under very dry or very wet conditions. However, soil drainage class had no effect.
- Increasing soil temperature increased the frequency of negative fluxes.
- The frequency of calculated negative fluxes was slightly higher when based on 3 headspace samples compared to 2 collected from chambers, suggesting fluxes based on 2 headspace samples does not increase the occurrence of negative fluxes.

6. Acknowledgements

We would like to thank Tash Styles for collating the Agresearch N_2O data. We also thank Janet Bertram, Roger Cresswell, Mandula Premartne and especially Rob Sherlock for valuable discussions. Tom Simpson contributed significantly to the GC precision measurements.

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Section 2

The potential for New Zealand pasture soils to act as nitrous oxide sinks – final report

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Landcare Research

Prepared for:

Lincoln University subcontract for a Ministry of Agriculture and Forestry – Sustainable Land Management and Climate Change (SLMACC) project

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ISO 14001

Contents

Executive Summary

Project brief

Landcare Research was subcontracted through Lincoln University for a Ministry of Agriculture and Forestry – Sustainable Land Management and Climate Change (SLAMCC) funded project to assess the potential for pasture soils to act as N_2O sinks. The project involved:

- Conducting a suite of tests for the GC systems used for measuring N_2O emissions.
- Using a systematic approach to investigate the analytical procedures involved in detection limits.
- Calculating a ± 95% confidence interval in order to determine the range of N_2O fluxes that can be detected given the GC detection limits.

Summary of objectives and reporting requirements

- Determine detection limits of GCs used for N_2O work, and associated error (confidence interval).
- Take the lower boundary of detection limit (determined above) and compare with datasets held to determine the incidence of 'real' negative fluxes, using data sets with urine, dung or control treatments.
- Summarise these data.
- Contribute to the final report by providing detection limits with associated errors and, a summary report of true negative flux occurrences (frequency and conditions prevailing).

Summary of results from the GC system at Landcare Research

- The calculated precision of N_2O gas analyses was 97.4%, which was independent of N_2O concentration of the gas samples tested.
- The mean absolute error (MAE) of the N_2O analyses was 40.8 ppbv N_2O, and this was also independent of N_2O concentration of the gas samples tested.
- The detection limit (DL) of N_2O concentrations using 500 ± 10 ppbv N_2O standard was 14.9 ± 1.1 ppbv N_2O.
- The 95% confidence interval for flux detection is approximately $2\sqrt{2}DL$ = 42 ppbv/hr or ~12 μg $N_2O/m^2/h$ for chamber (15 cm height) under standard conditions of temperature and pressure (STP).

A methane (CH_4) increase over a certain concentration (in this study 71.5 ppmv CH_4/hr) in gas collection chambers can falsely indicate af decrease in N_2O concentrations, resulting in apparent negative N_2O flux in chambers.

Of the 8000 flux measurements conducted over the last 10 years at Landcare Research a very small number (6) had negative fluxes greater than the 95% confidence interval for flux detection. These negative fluxes were measured only in a sheep-grazed pasture in the month of March.

An unresolved issue is whether or not the small levels of N_2O consumption that are observed infrequently represent a significant soil N_2O sink.

8. Introduction and Background

Soil can oxidise atmospheric N_2O and act as a N_2O sink. Soil consumption of N_2O as indicated by net negative N_2O fluxes has been reviewed under various conditions in both laboratory incubation experiments and in the field in natural and agricultural systems (Chapuis-Lardy et al. 2007; Kroeze et al. 2007). These reviews suggest that biological N_2O consumption in soil occurs through the conversion of N_2O to N_2 via denitrification. However, nitrifiers have also been reported to consume N_2O during nitrifier–denitrification (reduction of NO_2^- to NO and N_2O via N_2). Nitrogen limitation and soil wetness and temperature have been the key controls on soil N_2O consumption, while soil pH and O_2 content appear to be negatively correlated with N_2O consumption. For atmospheric N_2O to be consumed by soil it must diffuse into the soil, and this is only possible where the concentration gradient is favourable, i.e. soil N_2O concentration is less than the ambient atmospheric concentration. Chapuis-Lardy et al. (2007) concluded that, based on current knowledge, it is not yet possible to define clearly a set of conditions promoting negative N_2O fluxes. According to Billings (2008) very few field studies have reported net N_2O consumption at soil surface, and the small fluxes observed show no predictable relationship with soil moisture.

Chapuis-Lardy et al. (2007) consider that these small fluxes are often dismissed as an indication of the challenge of quantifying N_2O emissions near detection levels, rather than as robust evidence of soil N_2O consumption. Some researchers have reservations about attributing negative fluxes to oxidation, given other possible factors such as limitations in sampling methodology, detection limits, and analytical precision. As reported in some papers, and summarised in Saggar (2010), possible leakage due to poor seals or contact with soil may also result in the estimates of negative fluxes.

This project will provide information to assess the potential of New Zealand pasture soils to act as an N_2O sink by conducting a suite of tests comparing the analytical precision of the Landcare Research and Lincoln University GC systems, and then using a systematic approach to determine the detection limits of the GC systems and the incidence of negative fluxes from the available gas analyses data.

Objectives and reporting requirements

- Determine detection limits of GCs used for nitrous oxide measurement, and the associated error (confidence interval).
- Take the lower boundary of detection limit (determined above) and compare this with existing datasets to determine the incidence of 'real' negative fluxes, using data sets from experiments with urine, dung or control treatments.
- Summarise these data.
- Contribute to the final report by providing the detection limit and associated error, and a summary report indicating when true negative flux have occurred (frequency and conditions prevailing).

9. Suite of test for the GC

9.1 Methodology

A quality control campaign on measuring N_2O concentrations by gas chromatography (GC) systems at Landcare Research, Palmerston North, and Lincoln University was conducted during the last week of January 2011. A total of 200 gas samples ranging from 200 to 5800 ppbv N_2O (100 samples prepared by Landcare Research and 100 samples prepared by Lincoln University) were analysed in each of the labs (Table 1). In Landcare Research, Palmerston North, the gas samples were analyzed with a Shimadzu GC-17A system (Hedley et al. 2006).

Precision

The precision of a measurement system is the degree to which repeated measurements under unchanged conditions show the same results (Fig. 1). The coefficient of variation (C.V.) is a normalized measure of dispersion of a probability distribution. Here, we define the precision of the N_2O gas analyses by the Landcare Research GC system as follows:

Precision (%) = 100 – C.V. (%)

Accuracy

The accuracy of a measurement system is the degree of closeness of measurements of a quantity to that quantity's actual (true) value (Fig. 1). The mean absolute error (MAE) is a quantity used to measure how close measurements are to the true values (http://en.wikipedia.org/wiki/Mean_absolute_error). Here, we use the MAE to represent the accuracy of N_2O gas analyses from the Landcare Research GC system as follows (Equation 1) :

$$\mathrm{MAE} = \frac{1}{n}\sum_{i=1}^{n}|f_i - y_i| = \frac{1}{n}\sum_{i=1}^{n}|e_i|.$$

(Equation 1)

where, f_i is the measurement and y_i the true value.

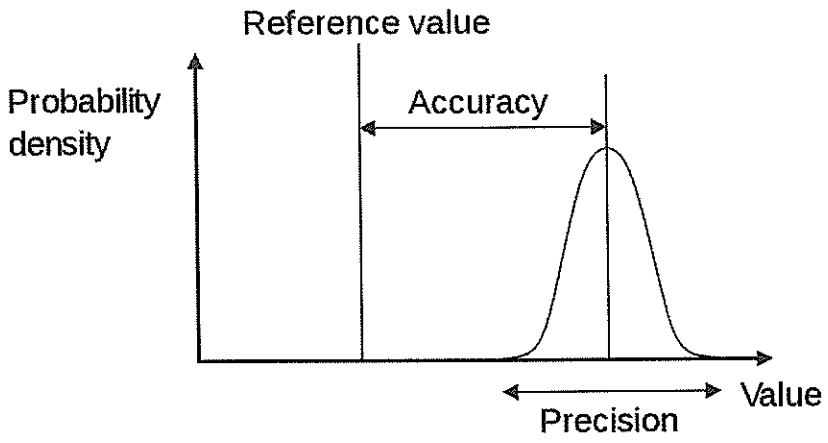

Figure 1 The concepts of accuracy and precision.

Accuracy indicates proximity of measurement results to the true value and precision indicates the repeatability or reproducibility of the measurement (http://en.wikipedia.org/wiki/Accuracy_and_precision).

9.2 Test results

The precision of N_2O gas analysis (range 200–5800 ppbv N_2O concentration) at Landcare Research was determined to be 97.4% (average; $N = 12$, minimum 93.8 to maximum 99.3%) (Table 1, Fig. 2). The precision was not significantly correlated with the N_2O concentration of the gases tested (Pearson correlation coefficient r = 0.504, $P = 0.10$), suggesting the precision of the Landcare Research GC system was not dependant on N_2O concentration of gas samples.

The MAE of the N_2O gas analyses (range 200–5800 ppbv N_2O concentration) at Landcare Research was determined to be 40.8 ppbv N_2O (average; $N = 12$, minimum 1.7 to maximum 160.1 ppbv N_2O) (Table 2, Fig. 3). The MEA was not significantly correlated with N_2O concentration of the gases tested (Pearson correlation coefficient r = 0.221, $P = 0.49$) suggesting the MEA of the Landcare Research GC system was not dependant on the N_2O concentration of gas samples.

Table 1 Nitrous oxide (N_2O) concentrations of prepared samples and N_2O concentrations determined on the Landcare Research GC system (Conc. is concentration, Aver. is average; S.D. is standard deviation; C.V. is coefficient of variation)

Designed N_2O conc.(A)	Determined N_2O conc.				
	N	Aver. (B)	S.D.	C.V*. (%)	100 – C.V. (%)
200	10	208.3	11.8	5.7	94.3
300	10	301.4	2.2	0.7	99.3
321.9	10	341.5	21.1	6.2	93.8
500	35	498.1	11.3	2.3	97.7
800	9	819.6	39.5	4.8	95.2
980	10	1098.9	15.9	1.4	98.6
1000	37	1019.4	27.4	2.7	97.3
1400	10	1385.9	26.9	1.9	98.1
1880	10	2040.2	37.3	1.8	98.2
2000	10	1972.5	31.6	1.6	98.4
2500	10	2509.2	18.5	0.7	99.3
5800	10	5759.9	51.8	0.9	99.1
Total	171			Aver.	97.4

*C.V. (%) = Aver./S.D. x 100

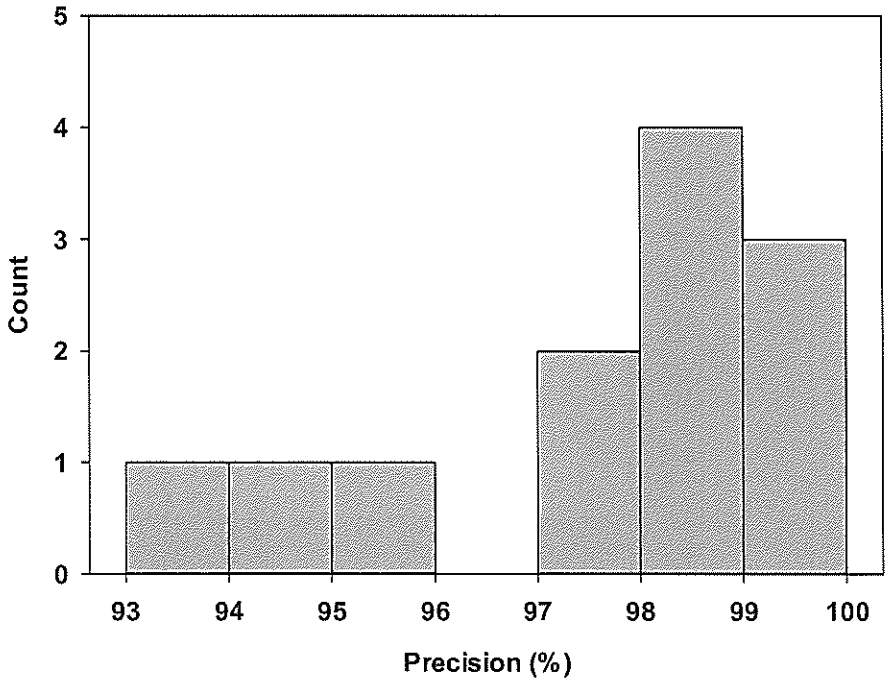

Figure 2 Histogram of the precision (%) of nitrous oxide (N_2O) gas analysis (range 200–5800 ppb N_2O concentration) using data from the Landcare Research GC system.

Table 2 Mean absolute error of nitrous oxide (N_2O) concentrations (range 200–5800 ppb N_2O concentration) from data produced by the Landcare Research GC system

N_2O concentration (N_2O ppbv)	Mean absolute error (N_2O ppbv)
200	10.5
300	1.7
321.9	19.6
500	9.4
800	26.6
980	118.9
1000	25.3
1400	18.2
1880	160.2
2000	31.5
2500	15.9
5800	51.4
Aver.	40.8

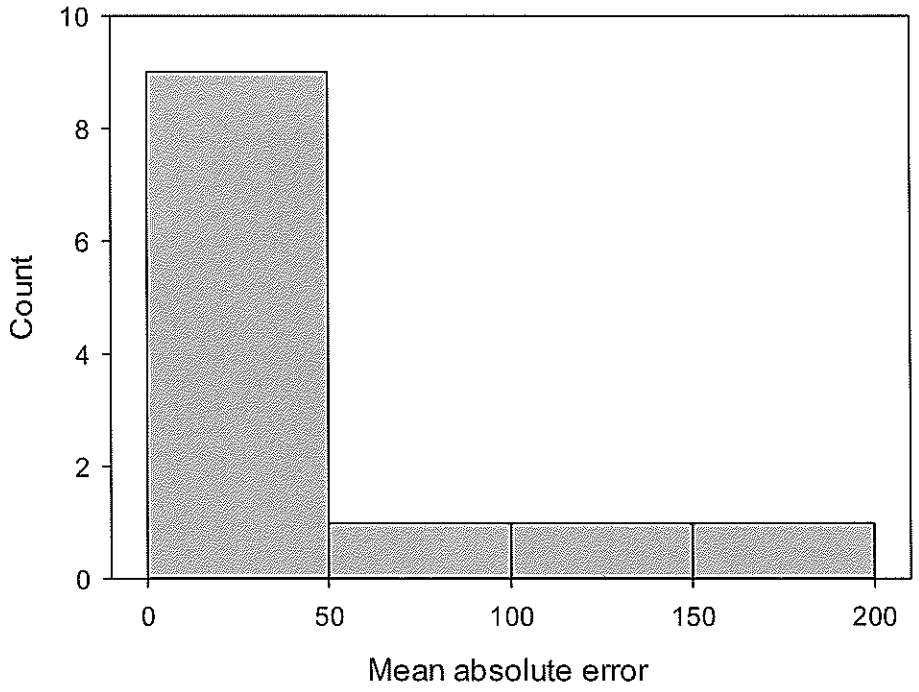

Figure 3 Histogram of the mean absolute error of nitrous oxide (N_2O) concentrations (range 200–5800 ppb N_2O concentration) from data produced by the Landcare Research GC system,

10. Detection limit of the Landcare Research GC system

10.1 Methodology for determining detection limit of N_2O analysis

During 2002 and 2010, the laboratory at Landcare Research, Palmerston North, used two different gas chromatographs (GCs) (Shimadzu GC-17A and GC-2010, hereafter referred to as GCI and GCII, respectively) to measure nitrous oxide (N_2O) concentrations in gas samples periodically collected from static chambers installed in field experiments to determine N_2O fluxes. A protocol was established to ensure the analytical precision and quality control involved using N_2O standards after every 20–30 gas samples, and two air samples collected from the field during gas sampling. The N_2O standards used during this period were either supplied by a commercial gas company (BOC Gases Australia Ltd.; 500 ± 10 ppbv N_2O) or prepared in the laboratory by mixing different commercially available standards with inert N_2 (480 and 700 ppbv N_2O). The variations in N_2O concentration results of the GC analyses of the N_2O standards were then used to determine standard deviation (s.d.) of the GC reading. The determined s.d. value is referred as the detection limits for each GC run.

10.2 Detection limits

During 2002 and 2010 a total of 149 sets each containing about 100 gas samples were analysed on GCI and 37 on GCII. The detection limits for GCI based on the results of 149 datasets was 14.4 ± 1.1 (mean ± standard error) ppbv, and that for the GCII (37 datasets) was 16.7 ± 3.5 ppbv (Table 3). Comparing GCI and GCII, while GCI had a significantly smaller detection limit than GC II in 2009 (t-test, $P = 0.006$), their detection limits were not significantly different in 2010 ($P = 0.881$) (Table 3). Overall, the detection limit of both the GCs did not differ significantly ($P = 0.618$) (Table 3). The detection limits determined using two different sources of N_2O standard gas (commercial vs lab-prepared) were also similar ($P = 0.209$) (Table 4). Our data show that 51.6 % of the detection limits were below 10 ppbv N_2O and 26.3 % were in the range of 10 to 20 ppbv N_2O. Only a small proportion (<5%) of the detection limits were above 50 ppbv N_2O (Table 5 and Fig. 4). Overall, the detection limit of N_2O concentrations measured by GCI and GCII in the laboratory during 2002 and 2010 was 14.9 ± 1.1 ppbv (95% Confidential interval of mean 2.2 ppbv) (Table 3).

Table 3 Detection limits of N_2O analysis by two different gas chromatographs (GCI and GCII) used by Landcare Research, Palmerston North (N = the number of data sets used to determine the detection limit, and s.e. = standard error of detection limit among the data sets)

	GC I		GC II		P value
Year	N	Mean ± s.e.	N	Mean ± s.e.	
2002	20	9.5 ± 1.2	*	*	*
2003	61	16.8 ± 2.2	*	*	*
2004	*	*	19	6.1 ± 1.3	*
2009	29	12.3 ± 1.3	12	34.9 ± 8.5	0.006
2010	39	14.7 ± 1.2	6	13.8 ± 2.7	0.881
Sub-total	149	14.4 ± 1.1	37	16.7 ± 3.5	0.618
Total (GC I + GCII)	186	14.9 ± 1.1	95% confidential interval of mean: 2.2 ppbv		

*no data

Table 4 Detection limits of N_2O determined from the use of two different sources of N_2O standard gases (commercial and laboratory-prepared) used by Landcare Research, Palmerston North (N = the number of data sets used to determine the detection limit, and s.e.= standard error of detection limit among the data sets)

	Commercial standard gas		Lab- made standard gas		P value
	N	Mean ± s.e.	N	Mean ± s.e.	
Detection limits	9	13.4 ± 1.6	165	14.3 ± 2.3	0.209

Table 5 Distribution of detection limits determined from the standards data sets analysed by two different gas chromatographs (GCI and GCII) used by Landcare Research, Palmerston North between 2002 and 2010

Range of detection limits (N_2O ppbv)	Count	Percentage (%)
0–10	96	51.6
10–20	49	26.3
20–30	19	10.2
30–40	6	3.2
40–50	8	4.3
50–60	6	3.2
60–70	0	0.0
70–80	0	0.0
80–90	1	0.5
90–100	0	0.0
100–110	1	0.5

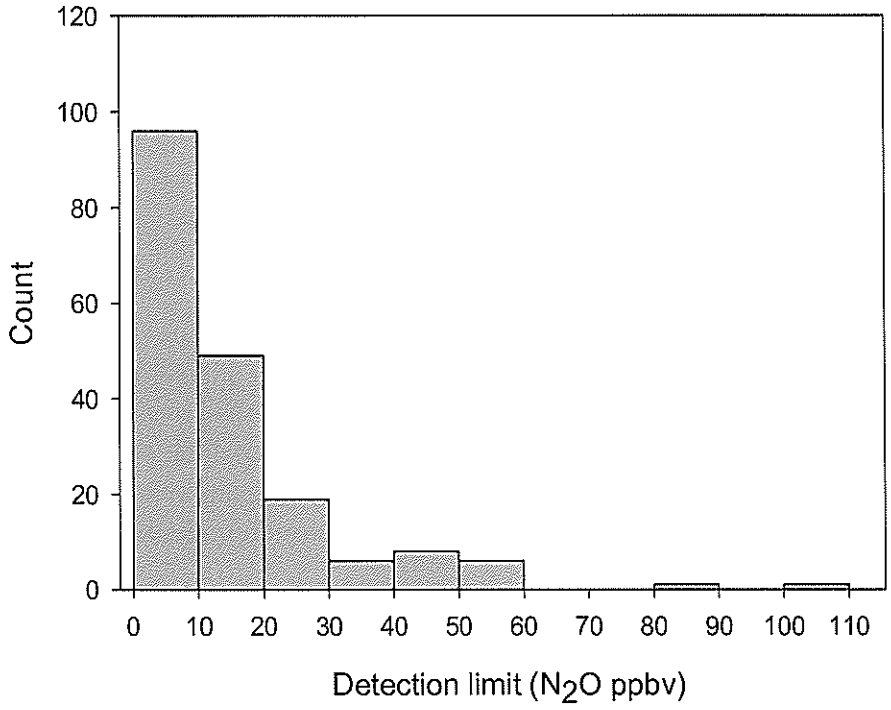

Figure 4 Histogram of the distribution of detection limits determined from the standards datasets by two different gas chromatographs (GCI and GCII) used by Landcare Research, Palmerston North between 2002 and 2010.

11. Occurrence of true negative fluxes

As shown above, the Landcare Research GC system has a detection limit (DL) of 14.9 ± 1.1 ppbv N_2O. As flux is the slope of concentration plotted against time the standard error of the slope (Equation 2) is approximately:

$$s_{flux} = \sqrt{DL^2 + DL^2} = \sqrt{2}D$$

(Equation 2)

According to Fig. 4, the distribution of detection limits does not appear to be normally distributed, but is positively skewed. However, we assume that the error in any particular reading followed a normal distribution for the purposes of determining a GC flux detection limit. So the 95% confidence interval for flux detection is approximately $2\sqrt{2}DL = 42$ ppbv N_2O /hr[1]. That is, there is only a 2.5% chance that the GC would underestimate a flux by more than 42 ppbv/hr.

Nitrous oxide measurement data were examined for 9 trials occurring between 2001 and 2010, which included over 8000 individual flux measurements. Apparent negative fluxes were eliminated from consideration if they did not meet the following criteria:

N_2O concentrations were measured at least 3 times to determine the flux.

The initial N_2O concentration was < 400 ppbv (as initial concentrations much higher than ambient could indicate effects due to soil disturbance).

The absolute value of the negative flux was greater than the 95% confidence interval for the GC flux detection (42 ppbv N_2O /hr for the Landcare Research GCs).

The change in N_2O concentration over time is linear with $R^2 > 0.9$.

There were no negative fluxes measured in dairy-grazed pastures. All the 8 negative fluxes that met these criteria were from a sheep-grazing study performed at Massey. Negative fluxes were in the grazed areas in March and September–October. However, the September–October results are most likely due to instrument error as the final concentration reading was close to zero, and soil moisture was at or slightly above field capacity. Out of the 6 remaining negative flux values 3 were observed from the 20 chambers on 20 March 2003. Soil moisture measurements were not made at the actual chamber sites. Mineral-N values were not available. Table 6 shows the negative fluxes and associated conditions.

[1] For a 15-cm-high chamber at standard temperature and pressure this would be equivalent to 12 μg $N_2O/m^2/h$.

Table 6 Negative fluxes detected at Landcare Research between 2001 and 2010

Site	Date	Chamber #	Treatment	Initial N_2O Concentration (ppbv)	Slope (ppb/h)	R^2	Flux (μg $N_2O/m^2/h$)	Rainfall in previous 24 h (mm)	Soil moisture 0–5 cm (vol %)
SBRU[2]	13/3/2003	14	Sheep grazed	383	–61	1.00	–17	0	13 ± 1
SBRU	13/3/2003	16	Sheep grazed	375	–64	0.94	–21	0	13 ± 1
SBRU	20/3/2003	13	Sheep grazed	371	–77	0.99	–19	0	10 ± 1
SBRU	20/3/2003	14	Sheep grazed	318	–44	0.93	–12	0	10 ± 1
SBRU	20/3/2003	16	Sheep grazed	353	–55	1.00	–18	0	10 ± 1
SBRU	25/3/2003	14	Sheep grazed	352	–49	0.93	–14	0	10 ± 2
SBRU	2/9/2003	17	Sheep grazed	346	–239	0.91	–67	0	59 ± 8
SBRU	2/10/2003	1A	Sheep grazed	337	–313	0.97	–75	0	68 ± 11

[2] Sheep and Beef Research Unit. Massey University

12. Influence of high chamber concentrations of methane on nitrous oxide concentrations

Methodology

Six closed chambers were used for this experiment. Gas samples (25 ml) were collected from the chambers and 25 ml of 0.2% CH_4 standard gas was then inserted in the chambers. This procedure was repeated 5 times. N_2O concentrations in the collected gas samples were analyzed by GC (Fig. 5).

Results

Detection limit of GC for N_2O gas

As shown above, the Landcare Research GC has a N_2O detection limit (DL) of 14.9 ± 1.1 ppbv and the 95% confidence interval for flux detection is approximately **Error! Digit expected.**42 ppbv N_2O /hr.

Effect of CH_4 concentration increase on N_2O concentration in chambers

Nitrous oxide concentration (vol/vol) significantly (P = 0.004) decreased in chambers following CH_4 gas addition (Table 7 and Fig. 6). A linear response of N_2O concentration to increasing CH_4 concentration was determined (Fig. 7, Equation 4), as follows:

N_2O concentration (ppbv) = $-0.5874 \times CH_4$ concentration (ppmv) + 274.85 (R^2= 0.9778)

(Equation 3)

Threshold CH_4 concentration caused a significant decrease in N_2O concentration in chambers

Using Equation 3 the minimum change of CH_4 concentration causing a decrease in N_2O concentration over flux detection (42 ppbv CH_4/hr) was determined as follows (Equation 4)

Minimum change of CH_4 concentration (ppmv)

ΔN_2O concentration = $-0.5874\ \Delta CH_4$ concentration

ΔCH_4 concentration (min. detectable) = $-42 / -0.5874 = 71.5$

(Equation 4)

When the CH_4 production in the chambers exceed 71.5 ppmv/hr, N_2O concentration will decrease above the detection limit (–42 ppbv N_2O /hr) in the chamber and, consequently, N_2O flux could be determined incorrectly as a negative value.

Our results suggest that an increase in CH_4 concentration in the gas collection chambers above a certain level (in this study, 71.5 ppmv CH_4/hr) can cause a false reduction in N_2O concentration and can therefore result in a negative N_2O flux estimate. These results suggest that gas sampling to determine N_2O flux in situations such as animal dung, where CH_4 flux can be high, needs to be interpreted with caution. In such a situation the following precautions should be taken: 1) the time during which the chamber is closed should be short to prevent increasing CH_4 concentration in chambers; 2) both CH_4 and N_2O concentrations must be analysed to check for the effect of high CH_4 flux. Further studies are needed to examine the effect of increasing carbon dioxide (CO_2), and increasing CO_2 plus, CH_4 concentrations on N_2O concentration and flux.

Table 7 Variation of methane (CH_4) and nitrous oxide (N_2O) concentrations in chambers (n=6) following CH_4 gas addition

Treatment	CH_4 (ppm, vol/vol)			N_2O (ppb, vol/vol)		
	Median	25%	75%	Median	25%	75%
Initial	2.3	2.3	2.3	273.8	271.9	275.3
1st CH_4 addition	6.6	6.5	6.9	270.6	269.7	270.9
2nd CH_4 addition	9.4	9.2	9.5	268.9	268.4	269.6
3rd CH_4 addition	11.5	11.5	11.6	268.3	267.7	268.8
4th CH_4 addition	12.8	12.7	12.8	267.6	266.6	269.1

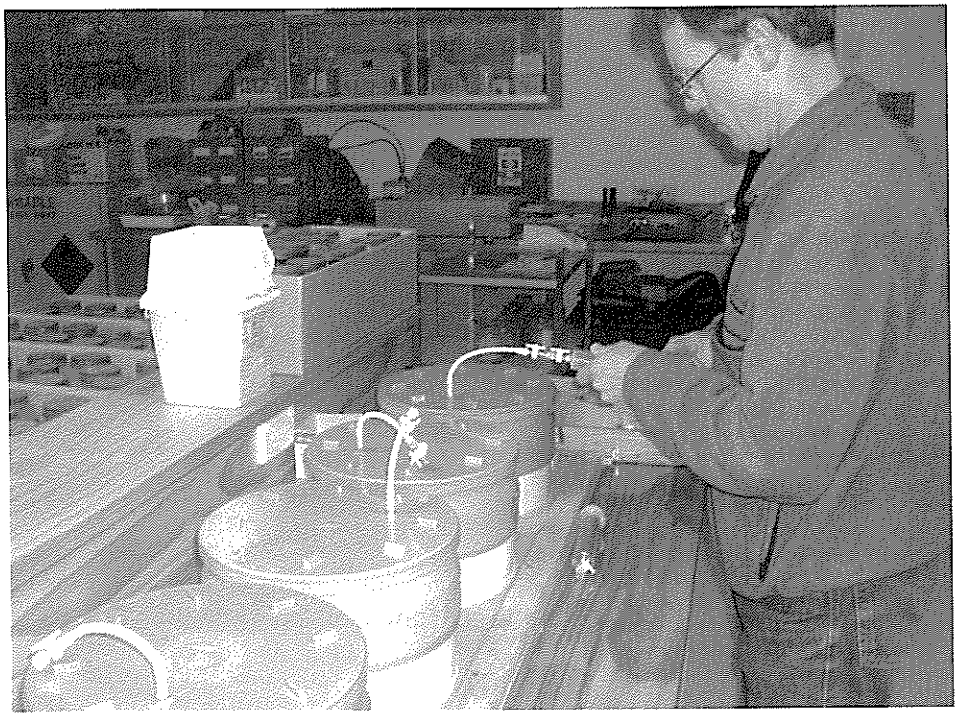

Figure 5 Gas sample (25 ml) was collected from 6 chambers, then 25 ml of 0.2% CH_4 standard gas was inserted in the chambers.

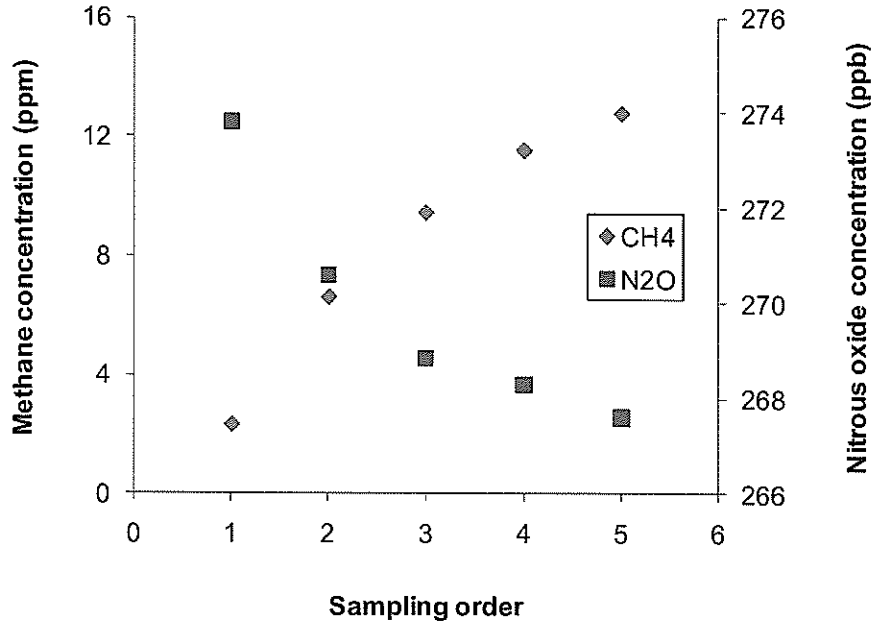

Figure 6 Variation of methane (CH_4) and nitrous oxide (N_2O) concentrations in chambers (n=6) following CH_4 gas addition. Methane was added before sampling for N_2O (from 2nd to 5th).

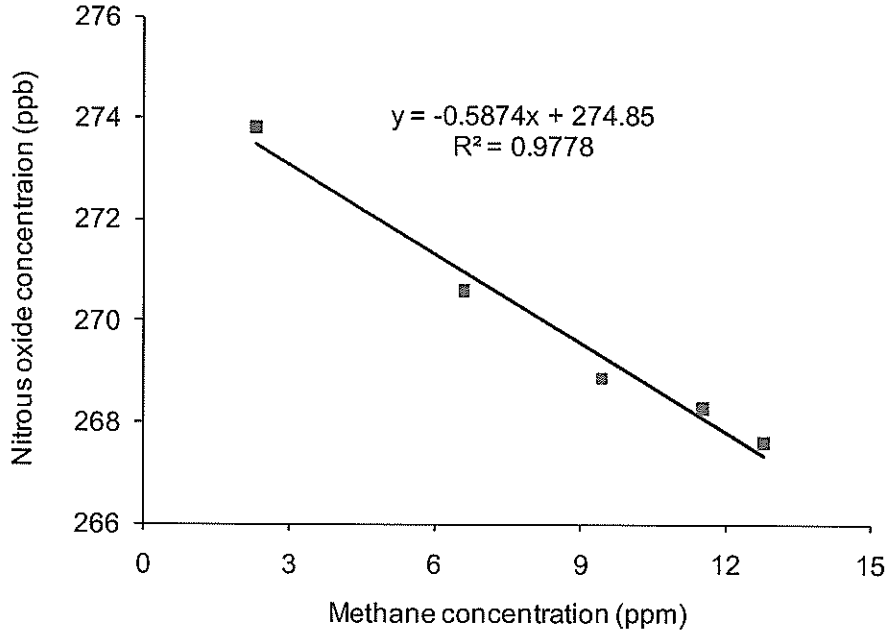

Figure 7 Relationship between methane (CH_4) and nitrous oxide (N_2O) concentrations in chambers (n=6) following CH_4 gas addition.

13. Conclusions

The data collected on N_2O analysis by the Landcare Research GC system over the past 10 years suggest that the precision of N_2O gas analysis was 97.4%, and the precision was independent of N_2O concentration in the gas samples tested. The mean absolute error (MAE) of N_2O gas analysis was 40.8 ppbv N_2O and the MEA was also independent of N_2O concentration in the gas samples tested. The detection limit (DL) of N_2O concentrations measured since 2002 using 500 ± 10 ppbv N_2O standard was 14.9 ± 1.1 ppbv and the 95% confidence interval for flux detection is approximately $2\sqrt{2}DL$ = 42 ppbv N_2O /hr or ~12 μg $N_2O/m^2/h$ for a 15-cm-high chamber at STP.

In a laboratory experiment we observed that increasing CH_4 over a certain concentration (that normally measured in dung treatments) in gas collection chambers (this study, 71.5 ppmv CH_4 /hr) can cause a false reduction in N_2O concentrations, resulting in negative N_2O flux in chambers.

Of the 8000 flux measurements conducted at Landcatre Research over the last 10 years on gas samples collected from agricultural, forest and shrub land soils and analysed in Landcare Research GCs, a very small number (6) had negative fluxes greater than the 95% confidence interval for flux detection. These negative fluxes were measured only in a sheep-grazed pasture in the month of March. An unresolved issue is whether or not the low levels of N_2O consumption that are rarely observed represent a significant soil N_2O sink.

14. Acknowledgements

The assistance of a number of technical staff in developing, testing and analysing standard and unknown gas samples in the two Landcare Research GC systems from 2001 to the present, Dr Kevin Tate for review, and Anne Austin for editing is gratefully acknowledged.

15. References

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Section 3

Comparison of Gas chromatograph analyses at Lincoln University and Landcare Maanaki Whenua.

Tim Clough, Frank Kelliher, Surinder Saggar.

This comparison was performed in order to assess if any bias was evident in the methodologies employed in the two laboratories. The occurrence of such a bias could, in theory, assist in explaining any observed trends for negative fluxes between laboratories.

Two 'blind' tests were performed. A series of gas samples were prepared in duplicate at Lincoln University (10 samples replicated ten times, a total of 100 samples) and analysed at both Lincoln and Landcare laboratories. A second series of gas samples (replicated 10 or 30 times) were prepared in duplicate at Landcare's laboratory and analysed at both Landcare and Lincoln.

Results of Lincoln sample analyses
At Lincoln University, N_2O concentrations included 0, 0.20, 0.32, 0.50, 0.98, 1.00, 1.88, 2.50, 5.80 and 45.00 $\mu l\ l^{-1}$ and these were were used in a 'blind' test. ALL gas samples were prepared from α or β gas standard bottles commercially prepared. No hand mixing or diluting of samples was performed. The N_2O standards were placed in 6 ml Exetainers® in a similar manner to routine gas sampling. Each gas standard was replicated 10 times, giving a total of 100 samples to analyse. This 100 sample batch was made up in duplicate with one batch sent 'blind' to Landcare, Palmerston North, and the other analysed 'blind' at Lincoln University.

Tables 1 and 2 show the results of the gas chromatograph analyses performed at Lincoln and Landcare, respectively. To see if the variability of the analyses followed any pattern with sample concentration the difference between actual sample concentration and the GC result (Lincoln known value minus the laboratory GC result) was analysed using ANOVA and Tukey's test.

Figure 1 shows the measured concentrations versus the known concentrations for both laboratory analyses of the Lincoln samples.

For the Lincoln analyses the Tukey's test showed there was no significant effect of N_2O concentration analysed on the mean difference (difference between the gas reference value and the analysis result) with a mean of this difference equalling -0.04 ± 0.29 (stdev). In the Landcare analyses there was no constant bias but there was an effect of N_2O concentration at 0.5 and 5.8 $\mu L/L$ with higher mean differences (Lincoln known value minus the Landcare GC result) and at 45 $\mu L/L$ where they were lower (Table 2).

Table 1 Lincoln analysis of 'Lincoln' standards which came from α or β gas standard bottles. Shown are the mean results for the 10 replicated analyses, the standard deviation, coefficient of variation, and the mean difference between the gas reference value and the analysis result (Lincoln known value minus the GC result).

N_2O standard ($\mu l\ l^{-1}$)	Analysis result ($\mu l\ l^{-1}$)	Standard deviation	Coefficient variation[1]	Mean Difference ($\mu l\ l^{-1}$)
0.00 (β)	-0.035	0.003	-8.19	0.035 *a*[2]
0.20 (β)	0.182	0.004	2.32	0.018 *a*
0.32 (α)	0.305	0.007	2.14	0.017 *a*
0.50 (β)	0.481	0.010	2.17	0.019 *a*
0.98 (β)	1.057	0.020	1.90	-0.077 *a*
1.00 (α)	0.981	0.010	1.04	0.019 *a*
1.88 (β)	1.996	0.055	2.77	-0.116 *a*
2.50 (β)	2.465	0.048	1.94	0.035 *a*
5.80 (β)	6.163	0.134	2.18	-0.363 *a*
45.00 (β)	45.006	0.866	1.92	-0.006 *a*

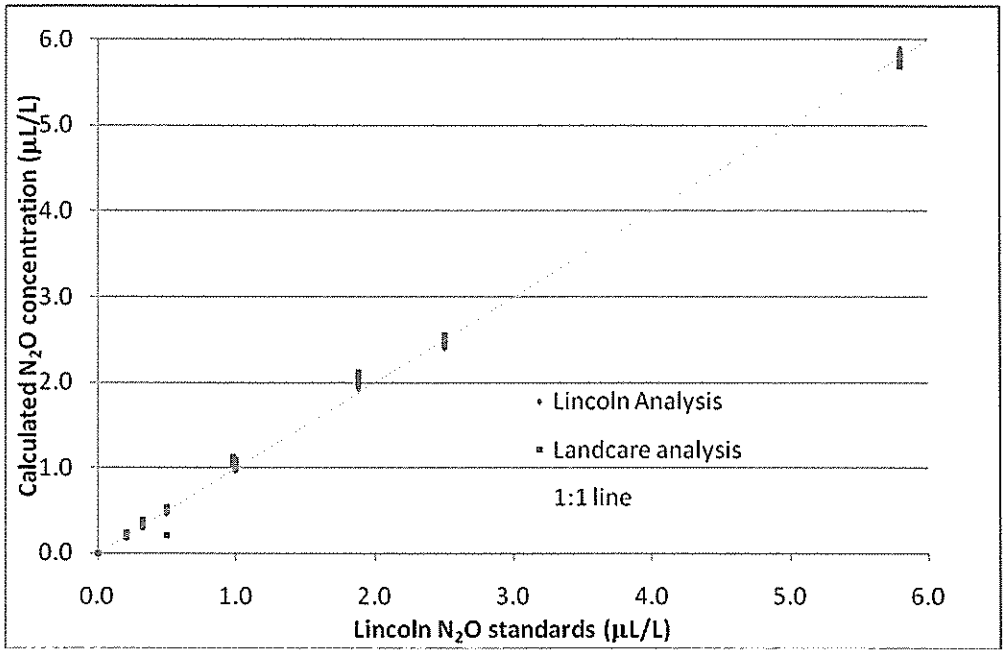
[1]Coefficient of variation is a measure of relative variability, equal to the standard deviation divided by the mean. [2]Tukeys test at 95% level of significance. Means that do not share a letter are significantly different.

Table 2 Landcare analysis of the 'Lincoln' standards which came from α or β gas standard bottles. Shown are the mean result of the 10 replicated analyses, the standard deviation, coefficient of variation, and the mean difference between the standard value and the analysis result (Lincoln known value minus the GC result).

N_2O standard ($\mu l\ l^{-1}$)	Analysis result ($\mu l\ l^{-1}$)	Standard deviation	Coefficient variation[1]	Mean Difference ($\mu l\ l^{-1}$)
0.00 (β)	0.000	0.000	*	0.000
0.20 (β)	0.208	0.0118	5.66	-0.008 *ab*[2]
0.32 (α)	0.341	0.021	6.17	-0.020 *ab*
0.50 (β)	0.424	0.148	34.85	0.076 *a*
0.98 (β)	1.099	0.016	1.45	-0.119 *ab*
1.00 (α)	1.057	0.010	0.97	-0.057 *ab*
1.88 (β)	2.040	0.037	1.83	-0.160 *ab*
2.50 (β)	2.509	0.019	0.74	-0.009 *ab*
5.80 (β)	5.760	0.052	0.90	0.040 *a*
45.00 (β)	45.752	1.669	3.65	-0.752 *b*

[1]Coefficient of variation is a measure of relative variability, equal to the standard deviation divided by the mean. [2]Tukeys test at 95% level of significance. Means that do not share a letter are significantly different.

Figure 1 Lincoln N_2O standards versus N_2O values determined in the Lincoln and Landcare laboratories. Excludes the 45 µL/L standard.



A twoway ANOVA with 'laboratory' and 'N_2O concentration'as factors showed that 'laboratory' had no significant effect (P =0.326) when determining the concentration of the Lincoln standards.

Results of Landcare sample analyses

In a reciprocal testing arrangement two sets of 100 evacuated vials were filled with N_2O standards at Landcare following routine sampling protocols to fill these. The N_2O concentrations were 0.30, 0.50, 0.80, 1.00, 1.40 and 2.00 µL L^{-1}, with replicate numbers of 10, 30, 10, 30, 10, and 10, respectively. The 0.50, 1.00 and 2.00 µL l^{-1} standards were 'bottled' with the other standards mixed by diluting with other non-N_2O gas.

Tables 3 and 4 show the results of the gas chromatograph analyses performed at Landcare and Lincoln, respectively. To see if the variability of the analyses followed any pattern with sample concentration the difference between actual sample concentration and the GC result (Landcare known value minus the laboratory GC result) was analysed using ANOVA and Tukey's test.

Table 3 Landcare analysis of the 'Landcare' standards which came from bottled or lab-mixed gas standards. Shown are the mean result of the 10 replicated analyses, the standard deviation, coefficient of variation, and the mean difference between the standard value and the analysis result (Landcare known value minus the GC result).

N_2O standard ($\mu l\ l^{-1}$)	Replicates	Analysis result ($\mu l\ l^{-1}$)	Standard deviation	Coefficient variation[1]	Mean Difference ($\mu l\ l^{-1}$)
0.30	10	0.301	0.002	0.71	-0.001 *a*[2]
0.50	30	0.459	0.132	28.72	0.041 *a*
0.80	10	0.762	0.187	24.56	0.038 *a*
1.00	30	0.938	0.228	24.29	0.062 *a*
1.40	10	1.386	0.027	1.94	0.014 *a*
2.00	10	1.973	0.032	1.60	0.028 *a*

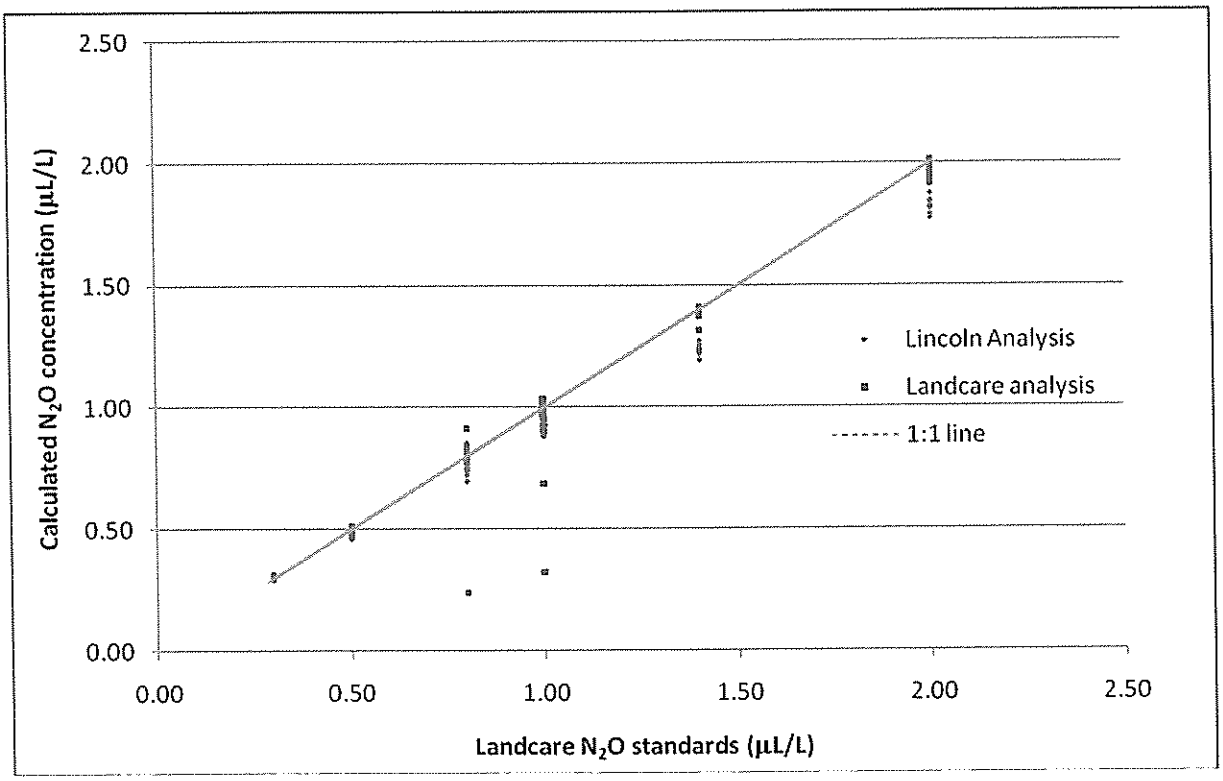
[1]Coefficient of variation is a measure of relative variability, equal to the standard deviation divided by the mean. [2]Tukeys test at 95% level of significance. Means that do not share a letter are significantly different.

Table 4 Lincoln analysis of the 'Landcare' standards which came from bottled or lab-mixed gas standards. Shown are the mean result of the 10 replicated analyses, the standard deviation, coefficient of variation, and the mean difference between the standard value and the analysis result (Landcare known value minus the GC result).

N_2O standard ($\mu l\ l^{-1}$)	Replicates	Analysis result ($\mu l\ l^{-1}$)	Standard deviation	Coefficient variation[1]	Mean Difference ($\mu l\ l^{-1}$)
0.30	10	0.299	0.006	2.04	0.001 *d*[2]
0.50	30	0.481	0.009	1.83	0.019 *d*
0.80	10	0.757	0.042	5.50	0.043 *c*
1.00	30	0.922	0.019	2.05	0.078 *b*
1.40	10	1.236	0.022	1.79	0.165 *a*
2.00	10	1.830	0.040	2.18	0.170 *a*

[1]Coefficient of variation is a measure of relative variability, equal to the standard deviation divided by the mean. [2]Tukeys test at 95% level of significance. Means that do not share a letter are significantly different.

Figure 2 Measured concentrations versus the known concentrations for both laboratory analyses of the Landcare samples.



For the Landcare analyses of the 'Landcare' standards the mean differences did not change with standard concentration ($p > 0.05$; Table 3). The Lincoln analyses of the 'Landcare' standards showed that the mean difference between the theoretically known value and the analysed values tended to increase with N_2O standard concentration ($p < 0.01$) as standard concentrations increased and differences were of the order 2.00, 1.40 > 1.00 > 0.80 > 0.50, 0.30 (Table 4). This can also be seen in Figure 2 where Lincoln analyses progressively underestimated the sample N_2O concentration as standard concentrations increased.

Because of the uneven replication of the standards a twoway ANOVA with 'laboratory' and 'N_2O concentration' as factors was performed for concentrations 0.5 and 1.0 µL/L where n=30. This showed no effect of 'laboratory' ($P = 0.904$) when determining the concentrations of the 0.5 and 1.0 µL/L Landcare standards.

Using twoway ANOVA again but this time looking at the remaining concentrations where n=10, i.e. 0.30, 0.80, 1.40, and 2.00 µL/L there was a laboratory effect ($P<0.01$) when determining the sample N_2O concentrations, with an interaction effect ($P<0.01$). This was predominantly due to the larger mean differences when determining the 1.40 and 2.00 µL/L

sample concentrations (Table 4 and Figure 2). At these 2 concentrations the Lincoln analysis underestimated the apparent sample concentrations.

Clearly there appears to be a bias in the analyses made. There is no simple way to determine which way the bias lies here, given there was no statistical bias observed with Landcare analysis of the Lincoln standards and there is no independent third party check of the standards made up and analysed in this cross lab check.

Some speculation on the cause could be considered e.g. incomplete pre-evacuation of the sample vial at Landcare laboratory prior to loading a sample would be a cause for increasing bias as sample concentrations increased. However, there is no obvious cause and parties should agree to a further cross check of samples and discuss routines and preparation procedures used.

There is an urgent need for comparison of these laboratories with a third or even a fourth laboratory.

Coefficients of variation attained in this laboratory comparison are on the whole (with the exception of Landcare analysis of Landcare standards 0.50, 0.80 and 1.0 μL/L) within the range described in sections 1 and 2 where the laboratories determined their respective precisions. This was ca. 2 % for the GC4 in the Lincoln laboratory and 0.9 to 6.2% for the Landcare laboratory. So while there is some bias or noise observed this *would not* be sufficient to contribute to negative fluxes being observed.

Recommendation

- Laboratories perform a cross-check of routines to establish any reason for bias seen in determination of Landcare samples. For example, cross- check procedures being used to: evacuate vials, load standards, and bring samples to ambient pressure prior to analysis.
- Establish a round-robin testing programme with external laboratories.

Section 4

N_2O concentration gradients in a soil profile

Tim Clough

Background and rationale.

For negative fluxes to occur there is a requirement for the N_2O concentration in the atmosphere above the soil surface to be greater than in the immediate soil profile, i.e. a diffusion gradient must exist (Clough, Sherlock and Rolston 2005). There is a dearth of studies where N_2O concentrations in the soil profile have been measured and/or followed over time. We are not aware of any such studies under pasture.

The objective of this study was to assess the potential for an N_2O diffusion gradient to form in a soil under pasture, by monitoring N_2O concentrations at a range of depths.

Materials and Methods

The rationale for site selection was based upon the need for a site that had received very little or no nitrogen inputs in the immediate past. The Lincoln University long-term ecology trial site was chosen because it had received no nitrogen fertiliser or excreta on the pasture area for 10 years. The area had a grass sward and was mown with clippings returned.

Stainless steel frames (1 m wide by 0.5 m deep by 0.02 m wide) were constructed with stainless steel wires horizontally spaced at 10 cm intervals. Silicon tubing (OD 18.7 mm, ID 12.5 mm) was attached to each of these steel wires with one end capped with a silicon suba-seal while the other end had attached, via a suba-seal, a stainless steel 3 mm tube onto which was placed a 3-way stop cock. This facilitated sampling of gas from within the silicon tube.

These replicated (n = 5) silicon assemblies were taken to the field site and carefully inserted in May 2011. This was achieved by digging a slot in the soil profile by hand and carefully separating soil as it was extracted. The silicon assemblies were lowered into position and the soil was hand packed so that the repacked soil resembled the original soil profile. The silicon tubes were placed so that the gas being sampled was centred on the 5, 15, 25, 35, and 45 cm depths. These silicon assemblies were left in the soil for several months to allow the soil to stabilise after its disturbance.

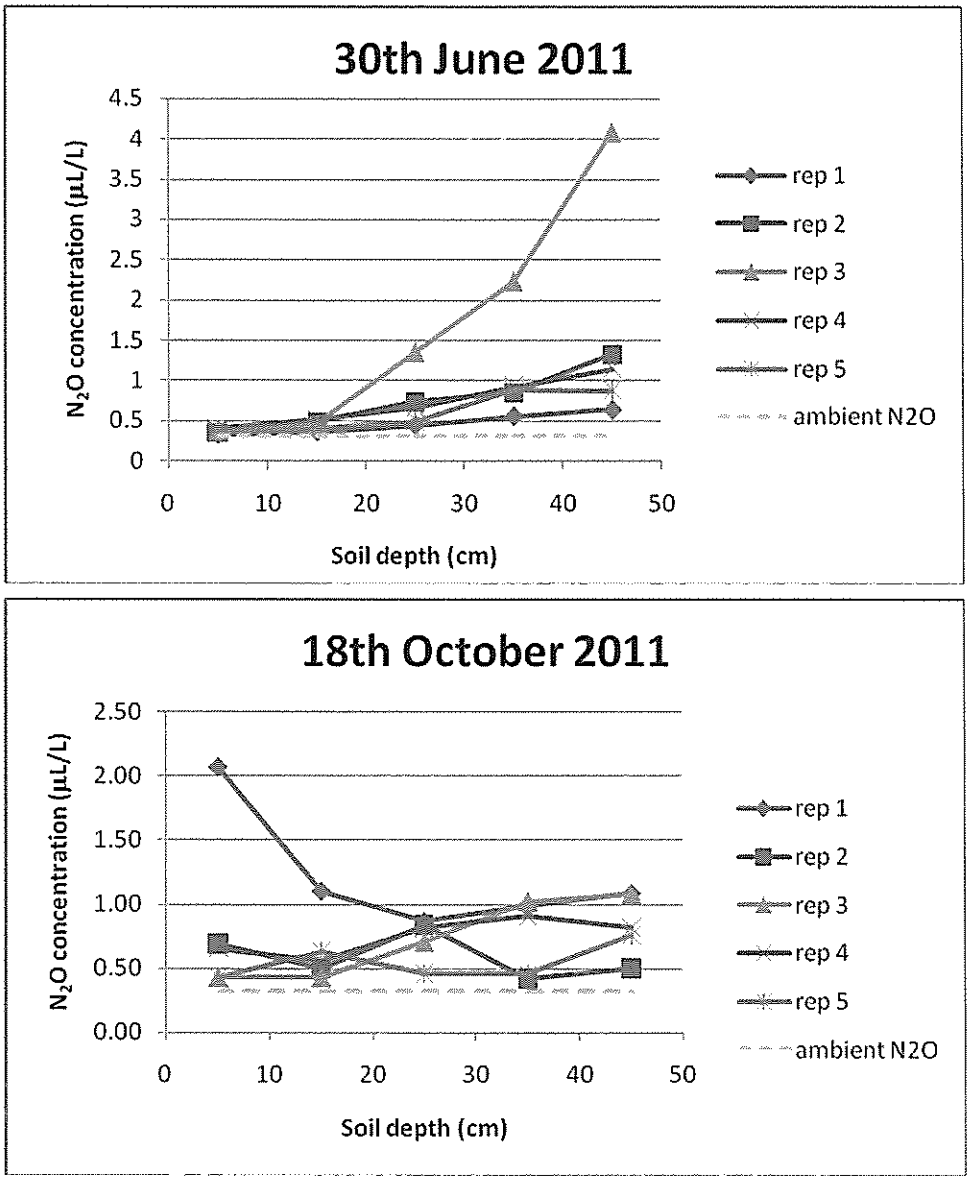
Gas samples for N_2O were taken in June 2011 and October 2011 from each depth when soil was at field capacity on both dates. A syringe equipped with a 3-way stop cock was attached to the silicon tube in question and a 10 mL gas sample was extracted and placed into a pre-evacuated 6 mL Exetainer®. These samples were then analysed at Lincoln.

Results and Discussion

It can be seen from the soil profile N_2O concentrations that the N_2O in the soil remains higher than ambient N_2O concentrations. This was regardless of the sampling time. Despite there being no nitrogen inputs to this site the disturbance of the soil may have lead to the release of

organic-N. This may have been subsequently transformed releasing N_2O, even several months after soil disturbance. So for this reason monitoring will be ongoing. What is apparent from the profiles is the increase in N_2O concentration with soil depth in June, and similarly in October with the exception of replicates 1 and 2 in October.

Figure 1 Changes in soil profile N_2O concentration with soil depth for two dates.



Clearly there is no N_2O diffusion gradient suitable for the uptake of N_2O at these times even though the N_2O concentration comes close to ambient (mean 0.36 ± stdev 0.03 µL/L) in June at the 5 cm depth.

These silicon tubes will remain in place and samples will be taken at future time points. Given the potential for negative fluxes to correspond with dry soil conditions it may be that a diffusion gradient could be established under summer conditions.

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Section 5

Assessing potential N_2O negative fluxes using a ^{15}N method.

Tim Clough

Background and rationale.
A previous study by Clough et al. (2007) used nitrous oxide (N_2O) labelled with the stable isotope ^{15}N, in combination with the conservative tracer sulphur hexaflouride (SF_6), to determine the fate of N_2O injected into ground water. In this study N_2O-^{15}N and SF_6, dissolved in previously collected groundwater, were reinjected into the groundwater and left to incubate in-situ. After the incubation period the water was re-extracted and the concentrations of the conservative SF_6 tracer and N_2O were measured and compared. By comparing changes in the ^{15}N enrichment of the N_2O, along with changes in the ratios of the initial concentrations to measured gas concentrations in the groundwater extracted, the fate of the N_2O-^{15}N added could be determined. A similar approach is used in the experiment conducted here. But instead of measuring gas concentrations in water the concentrations are measured in the chamber headspace over time. This has not been performed or attempted previously.

Table 1 summarises the possible scenarios following the injection of an N_2O-^{15}N and SF_6 mixture into a chamber headspace. For example, in scenario A if the initial ^{15}N enrichment of the N_2O is maintained over time it can be safely assumed that there is no in-situ production of N_2O from the soil adding to the headspace N_2O concentration, and if the ratio of C_t/C_o for N_2O behaves in an identical manner to the C_t/C_o ratio of the conservative tracer then there has been no consumption or loss of N_2O from the system at a rate greater than occurs with the SF_6 tracer. There could, however, be reduction of N_2O in the absence of any production and the C_t/C_o ratio of N_2O would decline faster than that of the SF_6 if this was the case and the ^{15}N enrichment of the N_2O would be constant (Scenario B).

If however, the N_2O ^{15}N enrichment decreases over time it means there is an ambient source of N_2O diluting the added N_2O, and if the ratio C_t/C_o of the N_2O increases with respect to the tracer then in-situ N_2O production is greater than any N_2O reduction rate (scenario C). Alternatively the ratio C_t/C_o of the N_2O with respect to the tracer may stay constant while the ^{15}N enrichment declines which indicates reduction and production rates are equal (scenario D), or the ratio may decline simultaneously with a decline in the ^{15}N enrichment indicating that production is less than the reduction rate (scenario E).

The implementation of such an experiment assumes SF_6 is biologically inert, and there is no evidence to suggest otherwise. The addition of N_2O into the headspace unavoidably increases the headspace N_2O concentration. It could be argued that this might slow the rate of diffusion from the soil surface IF there was no negative flux occurring. However, if a negative flux was occurring i.e. an N_2O sink, then a small increase in headspace N_2O concentration would

actually favour the sink process. So any artefact arising from increasing headspace N_2O concentrations would be beneficial in terms of trying to determine N_2O sink activity.

Table 1 Scenarios for changes in the headspace N_2O concentration and its ^{15}N enrichment.

Scenario	Initial N_2O-^{15}N enrichment	C_t/C_o for N_2O relative to SF_6 C_t/C_o	Potential reason for trends observed
A	Maintained	Conservative	No reduction[1] or loss of $^{15}N_2O$ and no in-situ N_2O production from natural abundance sources.
B	Maintained	Decreasing over time	Reduction of N_2O occurring with no in-situ N_2O production from natural abundance sources.
C	Decreasing with time	Increase over time	In-situ N_2O production from natural abundance sources greater than N_2O reduction rate.
D	Decreasing with time	Conservative	In-situ N_2O production from natural abundance sources equal to N_2O reduction rate.
E	Decreasing with time	Decreasing over time	In-situ N_2O production from natural abundance sources less than N_2O reduction rate.

[1]Here the term reduction refers to N_2O being reduced to N_2.

Materials and methods.

The experiment was performed at the Lincoln University long-term ecology trial area. No nitrogen fertiliser had been applied for over 10 years. Thus the rationale for choosing this area was a lack of fertiliser-N and urine-N history. The presence of which may have created either, N_2O emitting hot-spots or a higher turnover and production rate of organic matter derived-N.

The Wakanui silt loam soil was at field capacity when chamber bases were inserted, 1 week prior to adding treatments. Chambers were stainless steel, insulated with polystyrene foam to prevent internal changes in headspace temperature. Chambers had a diameter of 38.5 cm and their bases were inserted 10 cm into the soil creating a headspace of 11 L. Pasture inside and outside the chambers was 5 cm high during the experiment. Chambers were sealed when the chamber was placed onto the water trough that surrounded the chamber base.

An alpha standard cylinder of SF_6 gas (102 ± 2 μL/L in helium) was obtained from BOC gases. Standard curves were prepared and the level of detection using an electron capture

detector was < 0.01 μL/L. The intended concentration that would result following the injection of the tracer gas into the headspace was 0.5 μL/L.

Nitrous oxide was produced from ^{15}N enriched ammonium nitrate resulting in an N_2O mixture with a ^{15}N enrichment of ca. 2 atom% and a concentration of ca. 2300 μL/L which was diluted prior to it being injected into the headspace where it raised the ambient N_2O concentration by 2 μL/L and lead to an increase in the ^{15}N enrichment of the N_2O in the headspace. These were detectable levels and while a higher concentration of N_2O could have been used this would potentially further perturb the system in favour of creating an N_2O sink, i.e. if the headspace N_2O concentration is significantly greater than the soil atmosphere concentration the diffusion gradient favours N_2O diffusion into the soil and creates an artificial sink.

Two treatments were set up consisting of four replicates each. In the first treatment no amendments were made to the headspace of the chambers and the soil N_2O flux was measured over time. In the second treatment both ^{15}N enriched N_2O and SF_6 tracer were injected into the chamber headspace. An injection with a total volume of 10 mL (equal to 0.09% of the headspace), containing the ^{15}N enriched N_2O and SF_6, was made at time 'zero' with the injection syringe pumped slowly several times to ensure mixing of the injected gases with the antecedent headspace gas.

Headspace gas samples were taken from all treatments at times 0, 25, 56, 86, 125, 154, 184, 214 minutes. Samples were taken for N_2O and SF_6 concentration determinations by taking a 10 mL sample from each headspace and injecting this into a pre-evacuated (-1 atm.) 6 mL Exetainer®. These were analysed for N_2O and SF_6 on a GC equipped with an electron capture detector using appropriate standards. In those treatments where ^{15}N enriched N_2O was injected a further 15 mL gas sample was taken and placed in a pre-evacuated 12 mL Exetainer® for analysis of N_2O ^{15}N.

Results.

Where no gases were injected into the headspace the N_2O concentrations increased over time from an initial average concentration of 0.34 μL/L at time zero to 0.56 μL/L at 214 minutes, a net increase of 0.22 μL/L (Fig. 1). Consequently the ratio of C_t/C_o (concentration at a time t relative to the concentration at time zero) increased throughout the measurement period to be an average 1.66 ± 0.13 (s.e.m) at 214 minutes (Fig. 2). This equated to a mean flux of 0.20 $\mu g/m^2/min$ (2.9 g/ha/d).

Figure 1. Nitrous oxide concentrations in unamended headspace treatment.

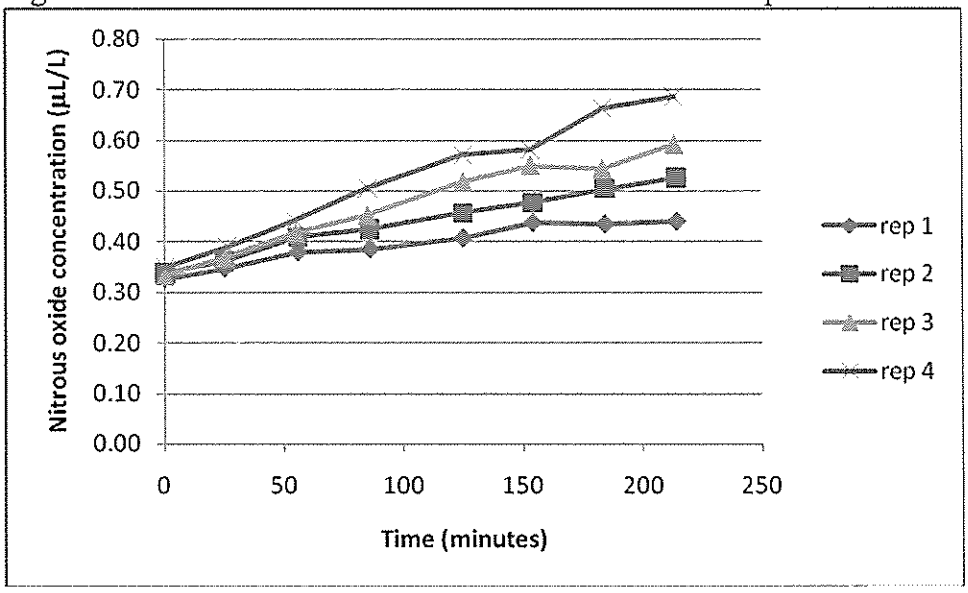
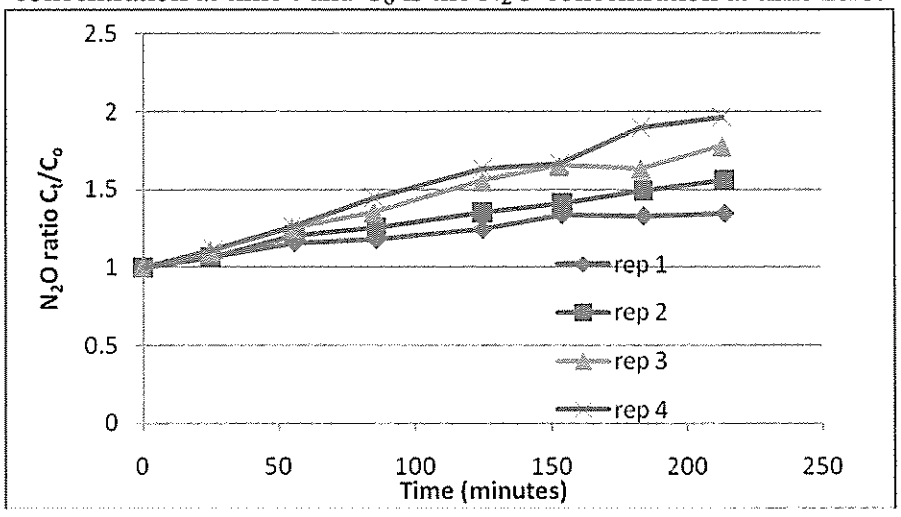
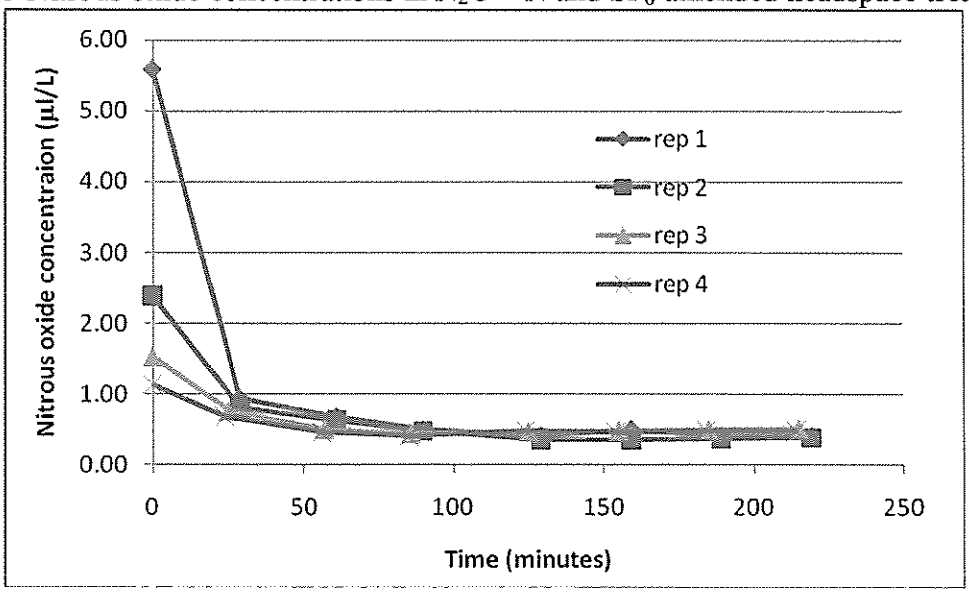


Figure 2. Ratio of C_t/C_o for N_2O in unamended headspace treatment. Where C_t is the N_2O concentration at time t and C_o is the N_2O concentration at time zero.



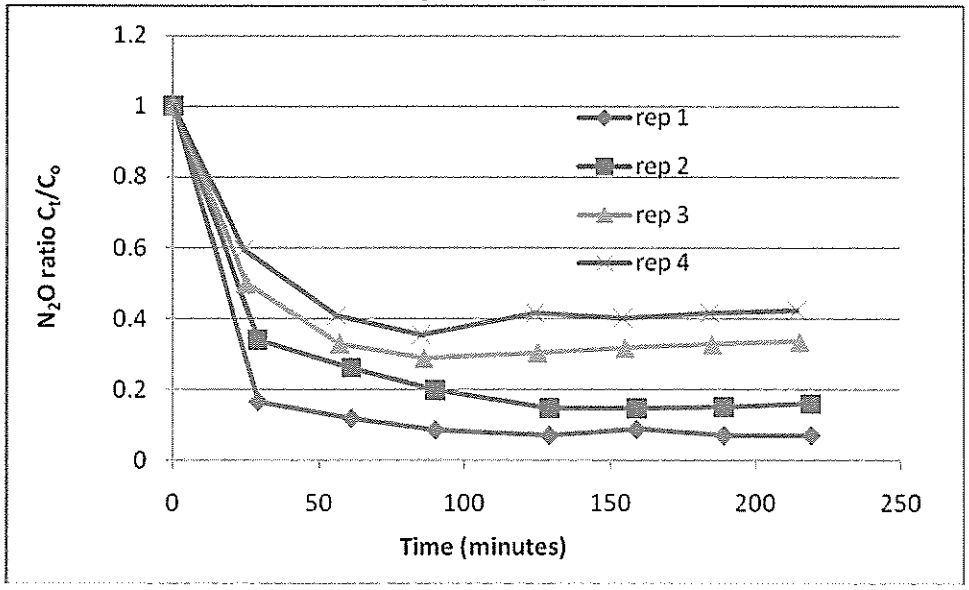
Where N_2O and SF_6 were initially injected into the headspace the initial N_2O concentrations averaged 2.34 ± 1.01 (s.e.m) at time zero and then decreased to be 0.45 ± 0.02 µL/L at 86 minutes (possibly as a result of mixing – see discussion below) after which they stabilised with three of the replicates having increasing N_2O concentrations between 96 and 214 minutes. However, the overall average at 214 minutes was 0.44 ± 0.03 µL/L.

Figure 3 Nitrous oxide concentrations in N_2O-^{15}N and SF_6 amended headspace treatment.



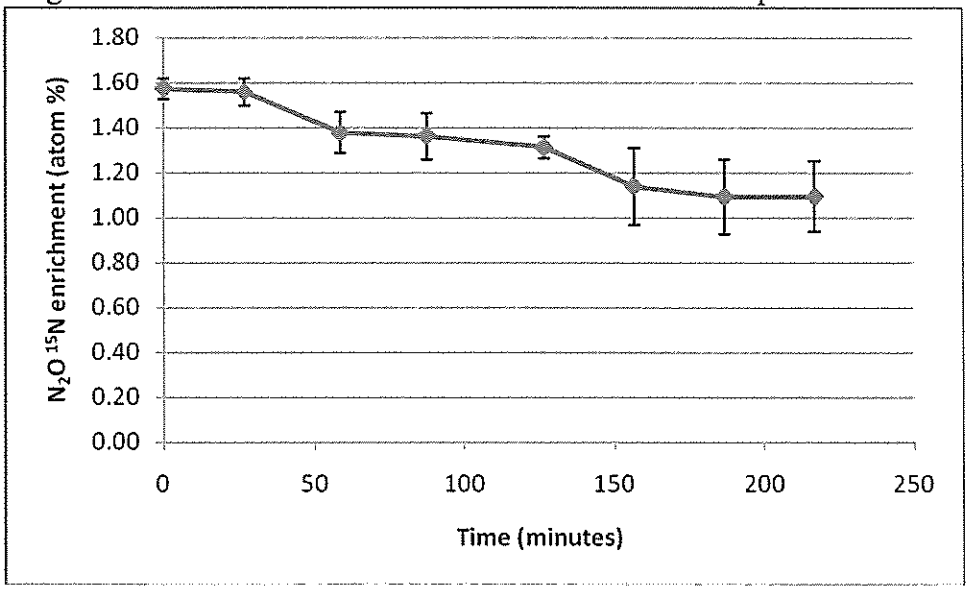
The ratios of C_t/C_o for N_2O in the headspace amended treatment reflected the initial decrease in N_2O concentrations and they declined rapidly until 86 minutes (Fig. 4). However, if this ratio was calculated for the period 129 minutes (as Co) to 214 minutes then values of Ct/Co ranged from 0.96 to 1.24 indicating N_2O concentrations increased during this period.

Figure 4. Ratio of C_t/C_o for N_2O in the headspace amended treatment. Where C_t is the N_2O concentration at time t and C_o is the N_2O concentration at time zero.



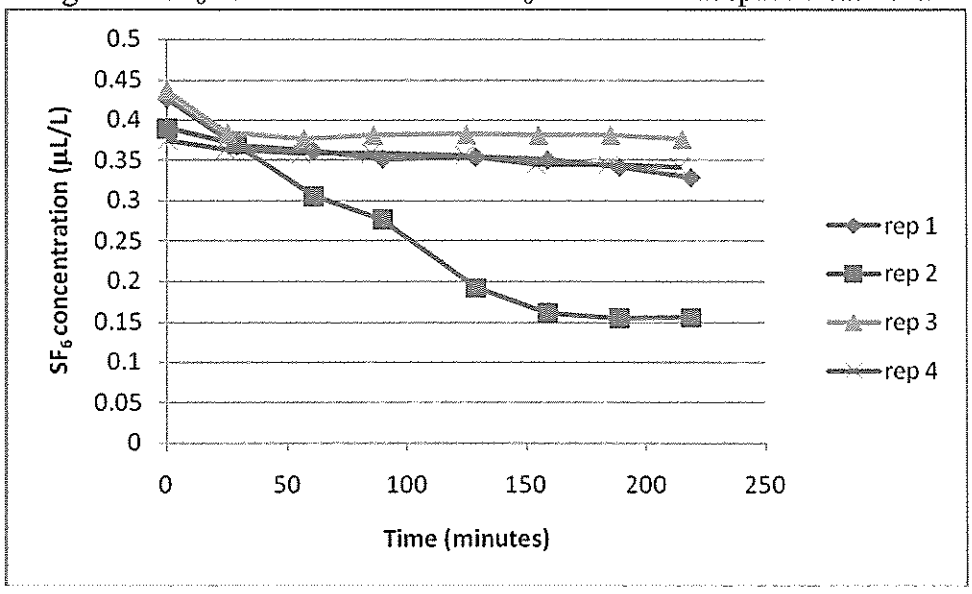
The ^{15}N enrichment of the N_2O in the headspace amended treatment averaged 1.57 ± 0.05 atom% at time zero and decreased steadily over time to be 1.09 ± 0.16 atom% by 214 minutes, indicating dilution from the in-situ production of N_2O (Fig. 5).

Figure 5. Nitrous oxide ^{15}N enrichment in amended headspace treatment



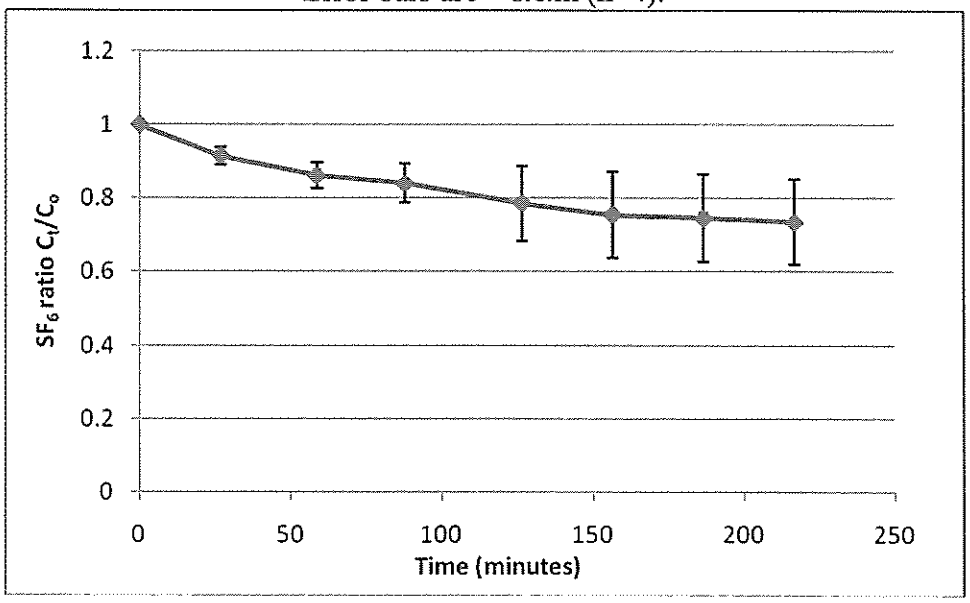
Concentrations of SF_6 averaged 0.41 ± 0.01 (s.e.m) μL/L at time zero and decreased to 0.30 ± 0.05 μL/L at 214 minutes. If replicate 2 was excluded the concentration at 214 minutes was still lower at 0.35 ± μL/L (Fig. 6).

Figure 6. SF_6 concentrations in the SF_6 amended headspace treatment.



As a consequence the ratio of C_t/C_o for SF_6 in the headspace amended treatment declined over time, equating to 0.74 ± 0.12 at 214 minutes (Fig. 7). Again if replicate 2 was excluded the value of the ratio at 214 minutes was 0.85 ± 0.04. The decline in the mean SF_6 C_t/C_o ratio correlated with the mean decline in the N_2O ^{15}N enrichment ($r = 0.95$).

Figure 7. Ratio of C_t/C_o for SF_6 in the headspace amended treatment.
Where C_t is the SF_6 concentration at time t and C_o is the SF_6 concentration at time zero.
Error bars are ± s.e.m (n=4).



Discussion and Conclusion

The unamended treatment (effectively the control) demonstrated that the soil and associated conditions were still able to generate N_2O despite a history of no N inputs from fertiliser or animal excreta. The average flux of 2.9 g/ha/d is comparable but at the lower end of the range previously reported for control plots in grazed pasture e.g. Clough et al. (2009) found N_2O fluxes from control plots ranged from 0.24 to 93 g/ha/d.

Given the soil was emitting N_2O, and N_2O concentrations continued to increase over time in the unamended chambers, the net N_2O concentration (N_2O injected + antecedent N_2O in chamber headspace + N_2O emitted from soil – N_2O taken up by soil) should have had a continual decrease in its ^{15}N enrichment over time. This is in fact what occurred (Fig. 5) with the excess ^{15}N enrichment (i.e. ^{15}N enrichment over and above the ambient value of 0.366) decreasing from 1.21 to 0.73 atom% ^{15}N. This is an effective dilution of 40% ((1.21-0.73)/1.21 *100/1).

The N_2O concentrations at time zero in the amended chambers presented a confusing picture at time zero, with a wide range of concentrations and concentrations higher than expected given the N_2O added. The explanation for this is almost certainly inadequate mixing of the headspace following N_2O addition. By 86 minutes mixing appears to have become more uniform and N_2O concentrations had stabilised and were increasing, with final concentrations averaging 0.44 µL/L. This is lower than seen in the unamended chambers but this is almost certainly a simple fact of the differences in individual replicates. One replicate in the amended chambers did have N_2O concentrations at 214 minutes equal to those seen in the unamended chambers. Future experiments of this type should trial the use of a fan, applied briefly, inside the chamber and to ensure rapid mixing close to time zero.

As noted above the N_2O measured in the headspace of the chamber is a net concentration which leads to the derivation of a net flux. Clearly a component of the N_2O measured in this experiment resulted from N_2O production. The fact that N_2O concentrations were increasing in the unamended chambers, and in the amended chambers in the final phases of the experiment, shows that the net flux was positive i.e. there was no net sink.

However, having stated this there was an apparent loss of N_2O from the amended chambers where N_2O was injected. The conservative tracer SF_6 provides information on physically induced gas loss. This could potentially occur as a result of diffusion into the soil, diffusion into the water seal, or a leaking chamber. It was observed that SF_6 concentrations decreased. The SF_6 C_t/C_o ratio indicated that the decrease in headspace SF_6 after 214 minutes was some 26%, or 15% if excluding replicate 2. While unmeasured it is expected that this was the result of SF_6 diffusing into the soil since SF_6 is absent in situ and the diffusion gradient favoured diffusion from the high headspace concentration into the soil profile.

The two gases, SF_6 and N_2O, have similar diffusion and water solubility characteristics and N_2O will have behaved in a similar manner to SF_6. Thus if the headspace N_2O concentration was higher than in the soil profile, which it was following injection of N_2O, then diffusion of N_2O into the soil would occur. This explains the initial decrease in N_2O in the amended chambers. The results indicate (Fig. 3) that it took about 86 minutes for the N_2O concentrations to adjust and for concentrations to again begin to increase. When gas concentrations above ground and at a given point in the soil profile are in equilibrium the term 'compensation point' is often used. Thus after 86 minutes a compensation point was reached.

In conclusion the results of this experiment demonstrate that there was no sink activity. The ^{15}N enrichment of the N_2O decreased over time, and post 86 minutes the Ct/Co ratio for N_2O increased relative to the conservative SF_6 Ct/Co ratio. Thus scenario the results favour scenario C where in-situ production of N_2O is greater than any sink activity.

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Section 6 Summary

Negative fluxes have previously been reported in the literature. For example, Flechard et al. (2005) frequently measured sub ambient N_2O concentrations in the soil profile which resulted in the measurement of net uptake fluxes of N_2O on an extensively managed grass sward. This was achieved using automated chambers with a flux detection limit given as 16 ng $N_2O/m^2/s$ while negative fluxes were of the order of 1 to 20 ng $N_2O/m^2/s$. So the bulk of these flux measurements were within the detection limit of the equipment used. Kroeze et al. (2005) reviewed 5 grassland studies where negative N_2O fluxes had been reported and the bulk of these were $\leq$0.8 ng $N_2O/m^2/s$ and again these were within the detection limits of the equipment used or detection limits were not stated.

Factors controlling N_2O uptake are those that control or favour N_2O reduction by denitrifiers: low inorganic N availability, soil moisture (anaerobicity), soil temperature, and optimal pH (Kroeze et al., 2005). It has been concluded that agricultural soils (usually fertilised) are not likely to be sinks for atmospheric N_2O (Kroeze et al., 2005).

However, N_2O fluxes can undoubtedly occur in agricultural soils if conditions meet the criteria noted above. The assessment of the AgResearch and Landcare data sets performed here in sections 1 and 2 represents one of the most comprehensive attempts to define the occurrence of negative fluxes. First by defining the detection limits of the GC the statistically significant limit for negative fluxes have been defined. Thus the negative fluxes 'beyond' this detection limit are valid.

Interestingly, in the AgResearch meta-analysis N_2O negative fluxes occurred more frequently when soil had $\leq$10 mg/kg NO_3-N, less water and when soil was warmer. Drier soils permit diffusion of N_2O further into the soil, while warmer temperatures increase rates of both gaseous diffusion and microbial reactions. Authors always seem intent on stipulating that drier soils are more aerobic, which they are, however, denitrification has been recorded in soils with aerobic profiles (e.g. Muller et al. 2004). What authors often fail to acknowledge is the fact that anaerobic micro sites occur even in anaerobic soils. These conditions, where negative N_2O fluxes were shown to occur more frequently, are consistent with the theory proposed by Kroeze et al. (2005)

Of interest here is also the higher frequency of negative N_2O fluxes observed in the presence of dung in the AgResearch trials. The only negative fluxes observed in the Landcare data set occurred under sheep grazing, possibly with dung present. Clearly further research is required to test the mechanism(s) and the potential occurrence of negative fluxes in dung affected soil. Is it because the environment is able to reduce N_2O more rapidly? Or is it an artefact potentially produced as the result of elevated methane levels under dung affected soil?

Few studies exist that have been specifically designed to evaluate negative sink occurrence. To perform such studies the equipment must be optimised and operating personnel must be at the top of their game. The lab comparison performed here produced good results but still leaves some questions to be answered and another lab comparison with a minimum of one extra independent laboratory is urgently needed to help find potential flaws in laboratory systems.

This work has also seen the instigation of silicon tubing installed to measure soil profile concentrations. This will be monitored over the coming summer months to assess if the soil

profile N_2O concentration does in fact drop below ambient levels. If no reductions are observed there is scope to impose further treatments such as a mowing height treatments or a dung treatment to try and stimulate a soil profile decline in N_2O concentrations.

For the first time a conservative tracer (SF_6) was jointly applied with a ^{15}N labelled N_2O gas into a headspace and their respective concentration declines and ^{15}N enrichments observed over time. While this was successful there is scope to improve upon the methodology in future. The experiment showed that under the conditions at the time no net negative flux of N_2O was occurring. Further experiments specifically targeted at determining negative flux rates could be undertaken and potentially need to be undertaken to resolve the issue and drivers of negative fluxes, particularly with respect to the question of dung.

In summary this work has shown negative fluxes can occur, albeit at a low frequency. Conditions driving these negative fluxes are hinted at from the meta-analysis. The magnitude of these negative fluxes and their significance in terms of offsetting N_2O emissions is unknown but would seem unlikely to be significant given the relatively low median values for negative fluxes, and the low frequency of their occurrence.

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