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## TANDEM THERMAL-CONDUCTIVITY AND ELECTRON-CAPTURE DETECTORS AND NON-LINEAR CALIBRATION CURVES IN QUANTITATIVE NITROUS OXIDE ANALYSIS

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(Received February 3rd, 1986)

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### SUMMARY

A gas chromatographic system with a thermal-conductivity detector and an electron-capture detector in series was designed to measure carbon dioxide and nitrous oxide evolved from soil at concentrations from ambient atmospheric levels to several per cent. The response of the electron-capture detector to different partial pressures of nitrous oxide was linear up to only 2.5 Pa. Electron-capture response to nitrous oxide at partial pressures up to 40 Pa was found to be closely approximated by representing nitrous oxide partial pressure as a fourth-degree polynomial function of peak area without an intercept. A two-point calibration method for adjusting the coefficients of the polynomial to compensate for day-to-day variations in electron-capture detector sensitivity is described.

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### INTRODUCTION

Losses of nitrogen from soils through denitrification are frequently measured using the acetylene-inhibition method<sup>1-3</sup>. In practice, this technique requires gas chromatographic measurement of nitrous oxide at concentrations from 0.03 to 100 Pa, a range too wide to be covered by any single type of detector. In many instances, samples will have to be diluted when an electron-capture detector is used, whereas for a thermal-conductivity detector the sample concentration of nitrous oxide often must be increased, for example by trapping the gas on a molecular sieve from a large volume. Neither solution is desirable when large numbers of samples are to be analysed.

The great spatial variability of denitrification rates, which gives rise to the wide range of nitrous oxide concentrations that must be analysed, precludes the calculation with statistical confidence of mean rates without considerable replication. The work described below was undertaken to produce a combination of physical apparatus and techniques of data analysis that would permit nitrous oxide to be accurately

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measured over a wide range of concentrations without pretreatment of the gas samples. In addition, it was desired that the same equipment used to measure nitrous oxide should be capable of performing simultaneous quantification of carbon dioxide and neon concentrations. Rates of carbon dioxide evolution are commonly used as indices of microbial activity in soil, and neon is frequently added to laboratory incubations for determinations of soil pore volume or the head-space of a container. A final requirement for the apparatus was that acetylene, which is added at 10 kPa to samples in the acetylene-inhibition method<sup>3</sup>, should not interfere with measurement of the gases mentioned above.

## EXPERIMENTAL

### *System design and operation*

Two identical systems (referred to hereafter as the front and back systems) were built in a Packard 428 gas chromatograph (Packard-Becker, Delft, The Netherlands). Each system consisted of a water filter, a Porapak QS column (Waters Assoc., Milford, MA, U.S.A.), a molecular sieve 5A column (Alltech Assoc., Deerfield, IL, U.S.A.), a thermal-conductivity detector (Packard-Becker) and an electron-capture detector (Packard-Becker) connected in series (Fig. 1; Table I). The gas flows were regulated for the front system (F) with the flow regulators of the gas chromatograph. For the back system (B), the flow was taken from the front one via a Tee union (Swagelok, Crawford Fitting, Solon, OH, U.S.A.) and restricted by a fine metering valve (Nupro, Willoughby, OH, U.S.A.). The reference gas for the thermal-conductivity detector was provided through a stainless-steel capillar (1.6 mm O.D.) and balanced with a restrictor (Nupro) to reach the detector at the same rate as gas from the column system (20 ml/min of helium). Stainless-steel tubing was used, and connections were made with Swagelok fittings. The water filters were  $\frac{1}{4}$  in. (O.D.) columns filled with anhydrous  $\text{Mg}(\text{ClO}_4)_2$  (technical grade).

The operation of the 8-port valves (Fig. 1) permitted gases within each system to flow in one of three modes. At the moment when a new sample left the sample loop (Fig. 2, arrow 1), carrier gas (helium) flowed from valve I, through the water filter and the Porapak column and was vented without restriction through valve II. Under these conditions, neon, nitrogen and oxygen travelled rapidly through the

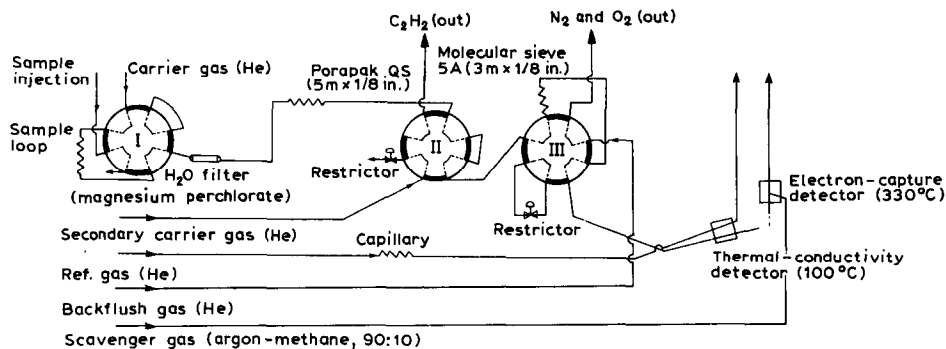


Fig. 1. Gas chromatography system for separation of neon, carbon dioxide and nitrous oxide in air containing acetylene. The system was duplicated in a Packard Model 428.

TABLE I

## SPECIFICATION AND OPERATING CONDITIONS OF GAS CHROMATOGRAPH

Model:	Packard 428.
Columns:	Two 5.0 m × $\frac{1}{8}$ in. O.D. stainless-steel, Porapak QS, 80–100 mesh columns. Two 3.0 m × $\frac{1}{8}$ in. O.D. stainless-steel, molecular sieve 5A columns. Oven temperature 45°C
Detectors:	Two thermal-conductivity detectors, Model 903; detector temperature 100°C; filament temp. setting 7 ( $\pm$ 350°C). Two <sup>63</sup> Ni electron-capture detectors, Model 902; constant detector current setting 8 (1.12 nA); temperature 330°C.
Carrier gas:	Main flow-rate (He): 20.1 (front) or 20.5 ml/min (back). Secondary flow-rate (He): 19.7 (front) or 20.4 ml/min (back). Backflush flow-rate (He): 20.3 (front) or 20.6 ml/min (back).
Scavenger gas:	Argon–methane (90:10), flow-rate: 40.5 (system front) or 41.0 ml/min (system back).
Sampling valve (I):	Carlo-Erba, Bimatic Model R, 1-ml sampling loop.
Venting valve (II):	Packard No. D 30915.
Backflush valve (III):	Packard No. D 30915.

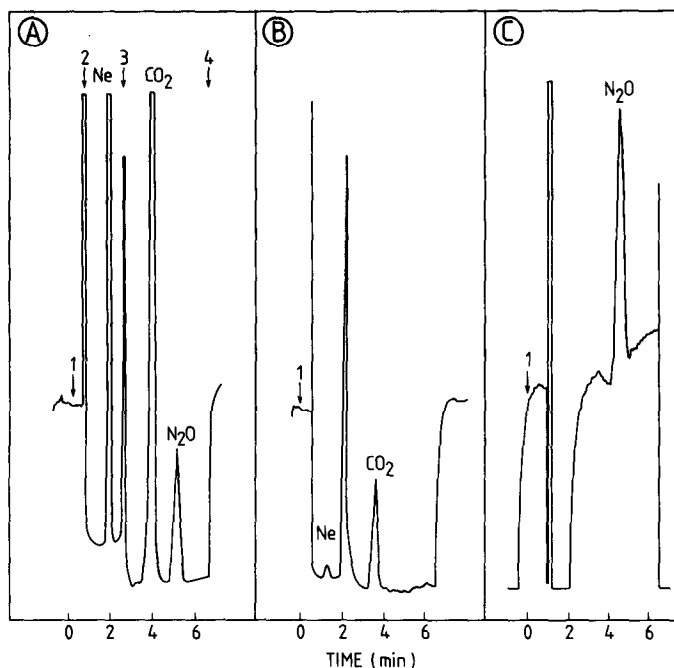


Fig. 2. Chromatograms showing separation of gases and detector sensitivities under conditions given in Table I. Arrows indicate switching of the 8-port valves as described in the text. Detectors and gases: A: neon (548 Pa), carbon dioxide (498 Pa) and nitrous oxide (116 Pa) by TC detection (front system); B: ambient neon (1.5 Pa) and carbon dioxide (33 Pa) from an air sample by TC detection (back system); C: nitrous oxide (0.0302 Pa) from the same sample as in B detected by an electron-capture detector connected in series with the thermal-conductivity detector.

column with little separation. To achieve better separation of neon (Fig. 2, arrow 2), the settings of valves II and III were altered 0.5 min after injection, so that the gas flow was from the Porapak column to the molecular sieve and finally through the detectors. While in this second mode, neon, nitrogen and oxygen came off the Porapak column and entered the molecular sieve. Neon reached the detectors before the next change in the gas flows, which occurred while nitrogen and oxygen remained in the molecular sieve and carbon dioxide, nitrous oxide and acetylene were still in the Porapak column. The system entered the third mode 2.0–2.3 min after injection (Fig. 2, arrow 3), at which time carrier gas from the Porapak column was shunted directly to the detectors over a restrictor, and the molecular sieve was simultaneously backflushed with helium supplied through valve III. Thus, carbon dioxide and nitrous oxide, which were well separated on the Porapak column, reached the detectors, and nitrogen and oxygen were flushed from the molecular sieve. Immediately after the nitrous oxide peak had cleared the detectors, the apparatus was returned to the first mode of operation (Fig. 2, arrow 4), and the resultant sudden drop in pressure in the Porapak column upon return to the first mode of gas flow resulted in rapid flushing of acetylene from the column. Irrespective of the mode of gas flow, the system was balanced so that helium reached the detectors at about 20 ml/min.

The detector signals were recorded on two double-pen linear recorders (Packard Model 621) and the signals were also integrated via four interfaces of a Sigma 10 Lab System (Perkin-Elmer, Norwalk, CT, U.S.A.). The 8-port valves were activated by air through 3-way solenoid valves (24 V, 2.5 W, Mass Magnett, Hannover, F.R.G.). The current was supplied by the Packard 428 and the valves were controlled by the Sigma 10 Lab System via relays within the interfaces.

Standard gas samples were diluted from pure nitrous oxide or a standard gas mixture containing 1160 ppm (v/v) nitrous oxide (Alfax, Malmö, Sweden) in serum bottles with rubber septa (Bellco, Vineland, NJ, U.S.A.) using 0.1–2.5 ml Hamilton syringes (Hamilton, Reno, NV, U.S.A.).

#### *Calculation of partial pressures*

The electron-capture detectors were used to measure nitrous oxide at levels above those at which the detector response was linear. To calculate the concentration of nitrous oxide in a sample from the area integrated under the nitrous oxide peak, the following empirical response-curve form was used:

$$N = P_s(A) = C_1A + C_2A^2 + C_3A^3 + C_4A^4 \quad (1)$$

where  $N$  is the partial pressure of nitrous oxide,  $A$  is the integrated peak area,  $P_s$  is a fourth-degree polynomial, and  $C_i$  are the coefficients of the polynomial. Polynomials are frequently used to approximate non-linear functional relationships of unknown form<sup>4</sup>. No intercept term was included in the model, because the area of the nitrous oxide peak was expected to be exactly zero when nitrous oxide was absent from a sample. The values of the coefficients,  $C_i$ , were obtained from fits of eqn. 1 by curvilinear regression<sup>4</sup> to a minimum of twelve values for peak areas and corresponding partial pressures of nitrous oxide spanning the range over which the electron-capture detectors were used.

When the non-linear portion of a response curve is used, a 1% error in peak area measurement will not result in a 1% error in calculated nitrous oxide partial pressure. To quantify the errors in calculated partial pressures, a response curve may be approximated at a particular peak area ( $A_1$ ) and nitrous oxide pressure ( $N_1$ ) by the line tangent to the curve:  $A = A_1 + m(N - N_1)$ , where  $m$  is the slope of the tangent. A small error,  $\Delta A$ , in measurement of a peak with area  $A_1$  will result in an error in calculated nitrous oxide pressure of  $\Delta N = \Delta A/m$ . The ratio  $(\Delta N/N_1)/(\Delta A/A_1)$  or  $A_1/(mN_1)$  expresses the relative sizes of errors as fractions of  $A_1$  and  $N_1$ . This ratio gives the factor by which an error in peak-area measurement will be reflected in an error in nitrous oxide pressure. For calculations of this ratio, here called the magnification factor, eqn. 1 was solved for  $N_1$ , and  $m$  was obtained as the inverse of the first derivative of eqn. 1.

### *Response-curve calibration*

Two different methods were examined for periodically calibrating the detector response curves to compensate for small changes in the sensitivity of the electron-capture detectors. The first method is based on the assumption that the shape of the response curve does not change; if a decrease in detector sensitivity results in a 10% underestimation of the amount of nitrous oxide in one sample, then the amounts in other samples will also be underestimated by 10%. To correct for this kind of change in the response curve, the area under the peak ( $A_1$ ), measured for a sample with a known partial pressure of nitrous oxide ( $N_1$ ), is used to calculate a dilation factor,  $d$ , from the formula  $d = N_1/P_s(A_1)$ . The standard polynomial is then dilated (or contracted) to produce the calibrated polynomial,  $P_c$ , by multiplying all the  $CV_i$  values by  $d$ . This calibration procedure will be termed the dilation method.

The second calibration method permits both the size and the shape of the detector response curve to change. With this method, both a dilation factor and a deformation factor,  $f$ , are used to modify the original polynomial according to the following formula:

$$P_c(A) = fA + dP_s(A) \quad (2)$$

The two factors,  $d$  and  $f$ , can be uniquely determined from two different measurements of peak areas,  $A_1$  and  $A_2$ , corresponding to two different partial pressures of nitrous oxide,  $N_1$  and  $N_2$ , as follows:

$$N_1 = fA_1 + dP_s(A_1) \quad (3a)$$

$$N_2 = fA_2 + dP_s(A_2) \quad (3b)$$

This linear system is solved for  $d$  and  $f$ , and these values are substituted into eqn. 2 to obtain  $P_c(A)$ . This type of calibration method will be termed the deformation method.

## RESULTS

The apparatus used in these studies gave clean separation of the three gases of

interest: nitrous oxide, carbon dioxide and neon (Fig. 2a). In addition, the separation between a 58-Pa nitrous oxide peak and a 10-kPa acetylene peak (not shown) was more than 0.5 min at the baseline. It was found that acetylene adversely affected the stability of the baselines and the sensitivities of the electron-capture detectors, but the good separation of the nitrous oxide and acetylene peaks permitted foreflushing of the latter from the Porapak column without any risk of losing a portion of the nitrous oxide peak. The electron-capture detector was sufficiently sensitive to detect nitrous oxide at partial pressure lower than ambient levels (0.03 Pa) in air (Fig. 2c), and the thermal-conductivity detector could measure partial pressure lower than ambient atmospheric concentrations of carbon dioxide (0.03 Pa) (Fig. 2b). Neon was accurately detected by the thermal-conductivity detector at 5.0 Pa (Fig. 2b).

The variation in precision of peak-area measurements using the thermal-conductivity and electron-capture detectors is shown as a function of the partial pressure of nitrous oxide in Fig. 3. At the lowest level of nitrous oxide determined (0.6 Pa), the integrators were usually unable to detect the existence of the nitrous oxide peak in the signal from the thermal-conductivity detector. Consequently, no coefficient of variation is shown in Fig. 3 for the thermal-conductivity detector at this partial pressure. At about 30 Pa and above, the coefficient of variation of the measurements from the thermal-conductivity detector ranged between 0.1 and 0.6% and did not appear to be strongly dependent on nitrous oxide partial pressure (Fig. 3). However, for nitrous oxide below 10 Pa, the coefficients of variation of thermal-conductivity measurements were greater than at the higher levels. In contrast, the variability of electron-capture measurements appeared to be independent of the partial pressure of nitrous oxide over the range tested (Fig. 3), and the coefficients of variation never exceeded 0.5%.

The response of the electron-capture detector to the partial pressure of nitrous oxide was approximately linear only up to nitrous oxide levels of about 2.5 Pa (Fig.

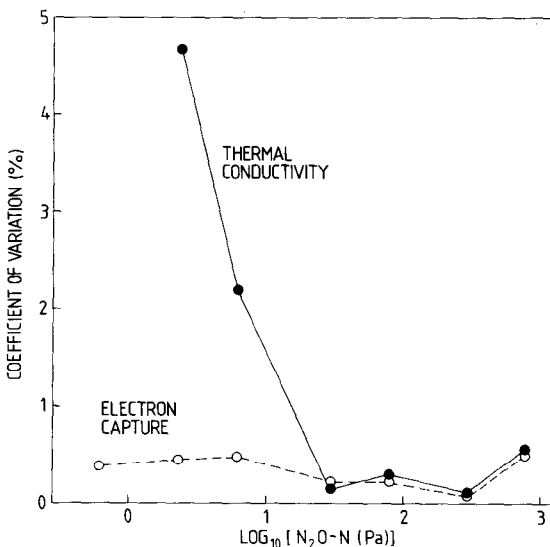


Fig. 3. The variability of the response of thermal-conductivity and electron-capture detectors as a function of nitrous oxide partial pressure ( $n = 6$ ).

4). The smooth curve (Fig. 4) is the fit of a fourth-degree polynomial (eqn. 1) to the data. The polynomial offered a close fit for partial pressures up to 40 Pa. The response of the thermal-conductivity detector was linear for nitrous oxide levels up to 1 kPa and is not shown. The broken lines in Fig. 4 illustrate the errors in calculated nitrous oxide levels that would result from 5% errors in peak-area measurement. A peak with an area of 180 integrator units lies slightly outside the linear region of the calibration curve, and a 6.3% error in partial pressure would be calculated as a result of the error in peak measurement. A similar error in measuring a peak with an area of 600 integrator units gives a considerably larger error (10%) in calculated partial pressure. The lower curve in Fig. 4 depicts the magnification factor calculated for the fitted calibration curve as a function of nitrous oxide partial pressure. Below 30 Pa, the magnification factor is less than 2.0, and a small error in peak-area measurement would result in the calculation of a nitrous oxide partial pressure that differs from the true value by a factor less than double the fractional error in measured peak area.

In order to use the electron-capture detector to measure nitrous oxide at levels above the linear response range, some method for periodical calibration of the non-linear response curve is necessary to compensate for small day-to-day variations in detector sensitivity. Two calibration methods have been described above. Both of these methods were used to recalibrate the response curve shown in Fig. 4 to relate nitrous oxide partial pressures to peak areas measured with a second electron-capture detector different from the first detector used to obtain the data for the original curve. The amount of nitrous oxide expected in thirteen samples (Table II, column 1) was calculated from the dilution of the standard gas used to prepare each sample. The response of the second electron-capture detector was measured for each sample. Use

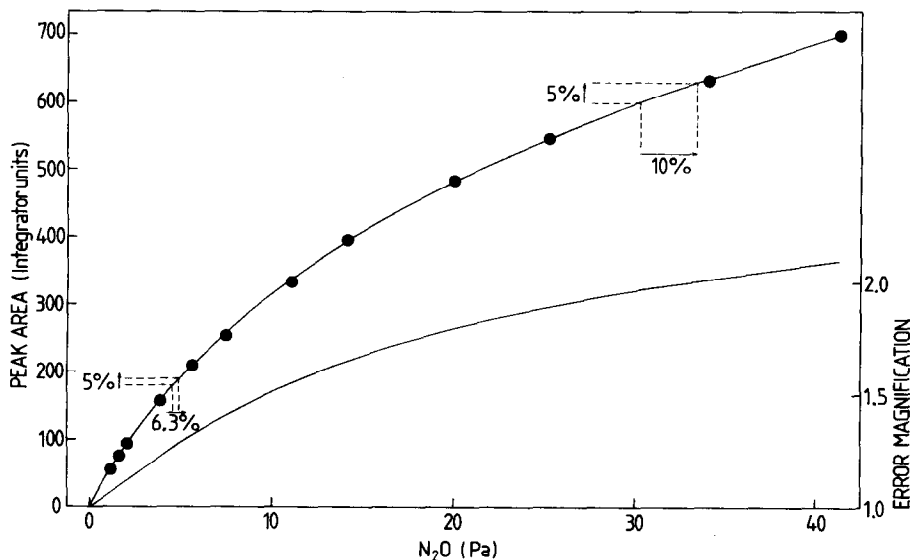


Fig. 4. The areas under nitrous oxide peaks measured with an electron-capture detector as a function of nitrous oxide concentration approximated by a fourth-degree polynomial. The dotted lines illustrate the magnification of errors in peak-area measurement resulting from the use of the non-linear region of the response curve to calculate concentrations, as explained in the text. The lower curve shows the magnification factor as a function of partial pressure.

TABLE II

ACTUAL PARTIAL PRESSURES (Pa) OF NITROUS OXIDE COMPARED TO THOSE CALCULATED FROM A POLYNOMIAL DETECTOR RESPONSE CURVE CALIBRATED USING TWO METHODS TO CORRECT FOR CHANGES IN DETECTOR SENSITIVITY

<i>Actual</i>	<i>Recalibration method</i>		<i>Actual</i>	<i>Recalibration method</i>	
	<i>Deformation</i>	<i>Dilation</i>		<i>Deformation</i>	<i>Dilation</i>
1.16	1.13	1.20	11.1	11.0	10.7
1.62	1.58	1.67	14.1	14.3	13.7
2.06	2.07	2.18	20.0	19.9	18.7
2.96	3.02	3.14	25.2	25.1	23.3
3.86	3.87	3.98	34.1	34.6	31.5
5.66	5.74	5.82			
7.46	7.46*	7.46*	41.3	41.3*	37.1

\* The sample was used for recalibration.

of the unmodified response curve shown in Fig. 4 resulted in an underestimation of the amount of nitrous oxide in all samples. The original response curve was then recalibrated using a procedure assuming that the amount of nitrous oxide was underestimated by the same percentage in all samples (the dilation method described above), and the modified curve was used to calculate the partial pressures (Table II, column 3). The other calibration technique (the deformation method), which permits the shape of the response curve to change, was also used to calculate the partial pressures (Table II, column 2). The amounts of nitrous oxide calculated using the dilation method exactly matched that expected in the sample with 7.46 Pa, because this sample was used to calculate the dilation factor used to modify the original response curve. Two samples (indicated in Table II) were used for the deformation method. The deformation method always predicted the nitrous oxide levels in the samples as or more accurately than the dilation method (Table II). At partial pressures of nitrous oxide much higher than in the sample used to calibrate the curve, the dilation method produced a response curve that underestimated the amount of nitrous oxide in the samples by up to 10%. In contrast, the deformation method produced a response curve giving the poorest prediction at the lowest nitrous oxide levels, for which the predicted and actual levels differed only by 3%.

## DISCUSSION

A 5-m Porapak QS column was employed principally to obtain a wide separation between nitrous oxide and acetylene. Adverse effects of acetylene on electron-capture detector stability and sensitivity have been reported previously<sup>5</sup>. This column length is at the upper end of the range of those reported to be routinely used for the separation of nitrous oxide from air samples<sup>6</sup>. Although the use of helium as the carrier gas would be expected to reduce the sensitivity of the electron-capture detectors below that which could be achieved using other carrier gases, operation of these detectors at 330°C may have compensated for this loss. Wentworth and Freeman<sup>7</sup> could detect ambient nitrous oxide concentration in air by operating their electron-capture detector at high temperature. Several investigators have used this detector at temperatures above 300°C to measure nitrous oxide at ambient concentrations<sup>8-11</sup>.



The coefficients of variation of measurements of nitrous oxide peak areas with the electron-capture detector were less than 0.5% over the entire range of partial pressures examined. Consequently, as an approximation, one would expect 95% of all measured areas to be within 1% (two coefficients of variation) of the correct value. The magnification factor was less than 2 for nitrous oxide levels less than 30 Pa for the response curve shown in Fig. 4. Therefore, if 95% of areas of peaks measured with the electron-capture detector are within 1% of the correct value, then 95% of the nitrous oxide partial pressures calculated from peak areas measured with the electron-capture detector should be within 2% of the true value (for nitrous oxide < 30 Pa). For nitrous oxide at partial pressures greater than 30 Pa, the coefficients of variation of peak areas measured with the thermal-conductivity and electron-capture detectors were approximately equal. For these high levels, greater accuracy is achieved by using the thermal-conductivity detector, because the response of the thermal-conductivity detector is linear and, thus, errors in peak-area measurement will not be magnified in calculations of partial pressure.

The coefficients of variation shown in Fig. 3 for the electron-capture detector are likely to reflect variation in the partial pressure of nitrous oxide in the sample loop as well as variability in the detector response and errors in peak-area integration. Errors in the amount of nitrous oxide introduced into the sample loop would not be expected to be magnified in pressure calculations in the same way as errors in peak-area measurement. In the absence of variability from other sources, a 1% increase in the amount of nitrous oxide injected would result in a (possibly) smaller increase in peak area. However, a 1% increase in the nitrous oxide pressure would be accurately calculated from that area provided that the fitted and the true calibration curves coincide over the range of areas concerned. The magnitude of the slopes of the fitted and true calibration curves would not affect the pressure calculation, as long as the slopes are identical (and non-zero). Given the accuracy of modern electronic signal integrators, magnification factors of the size encountered in this work would not appear to represent a serious deterrent for the use of the non-linear portions of calibration curves. The 95% confidence limit given above was calculated with the assumption that all errors would be magnified, and is probably an overestimate.

The response of the electron-capture detectors was linear with nitrous oxide partial pressure up to 2.5 Pa. A similar result was obtained by Kaspar and Tiedje<sup>11</sup>, who found that the response of their electron-capture detector was proportional to nitrous oxide at partial pressures less than 2.5 Pa. However, they reported that the shape of the response curve could be well modelled by two straight lines, the slope of the line for partial pressure above 2.5 Pa being slightly less than the slope for lower levels. In contrast, the response of the electron-capture detectors shown in Fig. 4 was well fitted by a model (a fourth-degree polynomial) in which the slope of the curve continuously decreased with increasing nitrous oxide partial pressure. Although a fourth-degree polynomial will not be applicable for all combinations of compounds and detectors, eqn. 1 offers a useful mathematical representation of the non-linear region of detector response curves resembling that shown in Fig. 4, permitting an extension of the range of concentrations which can be accurately calculated from integrated peak areas. It is hoped that the ease with which this model can be reliably recalibrated using the deformation method described above will encourage its use by future researchers.

During four years of routine use, the apparatus discussed in this paper was employed in the analysis of more than 45 000 samples. The large number of samples to be handled motivated many of the features of the design of the instrument. For example, the capacity of the apparatus was inexpensively doubled by duplicating the system (columns, valves and detectors) within a single machine. In addition, the electron-capture detector alone would have sufficed and it could have been used exclusively at concentrations for which its response was linear, if a reduction in the necessary number of analyses had afforded the luxury of diluting and reanalysing any samples found to contain more than 2.5 Pa of nitrous oxide. Given the number of samples, it was more efficient to avoid dilution and reanalysis by using an empirical mathematical function to extend the useful range of the electron-capture detector up to 30 Pa of nitrous oxide and to place a thermal-conductivity detector in series with the electron-capture detector to further extend the range of the instrument to 1 kPa.

#### ACKNOWLEDGEMENTS

The authors wish to thank Gun-Marie Ekström for typing the manuscript. Professor Thomas Rosswall and Jan Kristensson are gratefully acknowledged for critical reading of the manuscript. The Swedish Natural Science Research Council (NFR) provided financial support to T. Rosswall and L. Klemedtsson (Contract No. B-BU 4052-101). NFR also provided financial support to T. Rosswall and J. M. Tiedje jointly with NSF to allow S. Simkins to work at the department on a postdoctoral fellowship. The work formed part of the project "Ecology of Arable Land. The Role of Organisms in Nitrogen Cycling" financed by NFR, the Swedish Council for Planning and Coordination of Research (FRN), the Swedish Council for Forestry and Agricultural Research (SJFR) and the Swedish Environment Protection Board (SNV).

#### REFERENCES

- 1 W. L. Balderston, B. Sherr and W. J. Payne, *Appl. Environ. Microbiol.*, 31 (1976) 504.
- 2 T. Yoshinari and R. Knowles, *Biochem. Biophys. Res. Comm.*, 69 (1976) 705.
- 3 L. Klemedtsson, B. H. Svensson, T. Lindberg and T. Rosswall, *Swedish J. Agric. Res.*, 7 (1977) 179.
- 4 R. R. Sokal and F. J. Rohlf, *Biometry*, W. H. Freeman and Co., San Francisco, 2nd ed., 1981, p. 671.
- 5 B. H. Svensson, L. Klemedtsson and T. Rosswall, in H. L. Golterman (Editor), *Denitrification in the Nitrogen Cycle*, Plenum Press, London, 1985, p. 157.
- 6 A. R. Mosier and O. Heinemeyer, in H. L. Golterman (Editor), *Denitrification in the Nitrogen Cycle*, Plenum, London, 1985, p. 79.
- 7 W. E. Wentworth and R. R. Freeman, *J. Chromatogr.*, 79 (1973) 322.
- 8 R. A. Rasmussen, J. Krasnec and D. Pierotti, *Geophys. Res. Lett.*, 3 (1976) 615.
- 9 P. G. Simmonds, *J. Chromatogr.*, 166 (1978) 593.
- 10 A. R. Mosier and L. Mack, *Soil Sci. Soc. Am. J.*, 44 (1980) 1121.
- 11 H. F. Kaspar and J. M. Tiedje, *J. Chromatogr.*, 193 (1980) 142.