# The measurement of $^{15}N$ in soil and plant material

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**Abstract.** A complete procedure for analysing soil and plant samples for total N and atom % excess $^{15}N$ is described. The salicylic acid version of the Kjeldahl method for measuring total N was modified for use in a digestion block, giving quantitative reduction of nitrate in both soil and plant material. Procedures for minimising cross-contamination between samples are specified, including a double-distillation procedure that eliminates 'memory effects' when distilling $NH_3$ from Kjeldahl digests. A simple and robust apparatus for converting $(NH_4)_2SO_4$ to $N_2$ gas for mass spectrometric determination of atom % excess $^{15}N$ is described. The coefficient of variation for replicate measurements of total N in soil and plant material over the range 0.1–2.2% N was 1.0%. The coefficient of variation for measurements of $^{15}N$ in plant material over the range 0.4–2.9 atom % excess $^{15}N$ was 0.2%.

The Kjeldahl method is commonly used to measure the nitrogen content of soils, plants and other biological materials. Nitrogenous compounds are converted to the ammonium form by digestion, the digest treated with alkali, steam distilled to release ammonia gas, and the ammonia trapped and titrated against sulphuric acid [1, 4, 5]. If the $^{15}N/^{14}N$ ratio of the material is also to be determined, nitrogen in the resulting $(NH_4)_2SO_4$ is converted to $N_2$ gas by reaction with LiOBr. The ratio in the $N_2$ is then measured by mass spectrometry. Various techniques have been described for the conversion of $NH_4^+$ to $N_2$ [3, 11, 12]; in all of them a disposable glass vial containing the $(NH_4)_2SO_4$ is attached to a preparation line connected to the sample inlet of the mass spectrometer. After evacuation, LiOBr is run into the vial from a reservoir.

We describe a new method in which LiOBr is injected on to dry $(NH_4)_2SO_4$ in the sample vial through a rubber septum. This simplifies degassing and eliminates the need for complex glass apparatus, including a LiOBr reservoir, mounted above the mass spectrometer.

We also describe a modified steam distillation procedure, designed to eliminate $^{15}N$ cross contamination between samples, and give details of a salicylic acid modification of the Kjeldahl digestion process that is suitable for use with a heating block.



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## Experimental

*Sample preparation*

Plant and soil samples are ground in a disc mill (Tema model T100, with a 100 $cm^3$ capacity grinding chamber) for 2 minutes. This type of mill is preferable to a knife or hammer action mill as it can be thoroughly cleaned by washing between samples, thus eliminating $^{15}N$ cross-contamination. For satisfactory results with the Tema mill, plant samples must be ground immediately after drying at 80 °C. The harvesting, threshing, grinding and analysis of samples from a particular experiment is done, as far as possible, in order of ascending enrichment, so as to minimise cross-contamination problems, particularly between enriched and unenriched (control) treatments.

*Kjeldahl digestion*

The salicylic acid modification of the Kjeldahl method [1] is used for both plant and soil samples.

*Reagents.* Salicylic acid/sulphuric acid mixture. Fifty g AR salicylic acid dissolved in 2 litres AR concentrated sulphuric acid.
Thiotabs LR. Each tablet contains 5 g $Na_2S_2O_3 \cdot 5H_2O$.
Kjeltabs 1B. Each 5 g tablet contains 100 parts $K_2SO_4$, 6 parts $CuSO_4 \cdot 5H_2O$ and 1 part Se.
(Thiotabs and Kjeltabs supplied by Thompson and Capper Ltd., Liverpool).

*Procedure.* Sufficient sample to contain about 5 mg of nitrogen (e.g. 5 g soil, 1 g straw, 1 g roots, 0.5 g grass, 0.25 g grain) is weighed into a dry 250 ml graduated digestion tube. Forty ml salicylic/sulphuric acid mixture is added and left overnight at room temperature. One Thiotab is then added and the tube swirled occasionally until frothing ceases. Two Kjeltabs are added and the tubes placed in a 20 place aluminium digestion block (Gerhardt Kjeldatherm Block KT20). The tubes are heated for 3 hours (from cold) at 360 °C. After cooling the digests are made up to volume with distilled water.

Samples are analysed in duplicate and two reagent blanks included with each batch of 19 samples. A standard grass sample containing 1.407% N and with an atom % excess $^{15}N$ of 0.4156 is analysed at frequent intervals; results within 3 standard deviations of those given in Table 4 are taken as acceptable.

*Distillation of Kjeldahl digests*

*Reagents.* Sodium hydroxide solution 10*M*.
Sulphuric acid, 0.025*M* standardised against the ammonia produced by steam distilling a known amount of AR $(NH_4)_2SO_4$ with NaOH.
Boric acid solution, 2% W/V in distilled water.

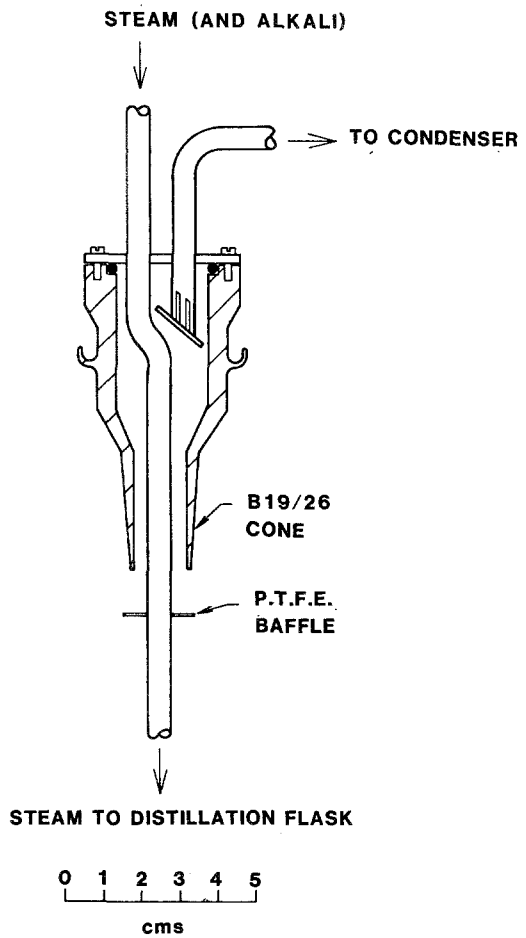


Figure 1. Construction of the distillation head and trap used for steam distilling $NH_3$ from Kjeldahl digests. All parts except the P.T.F.E. baffle and the O-ring are of stainless steel.

*Apparatus.* Aliquots of the digest are steam distilled using a modification of the steam distillation apparatus described by Bremner for measuring inorganic forms of N [2]. Rather than the all-glass system used by Bremner, the condenser and spray trap (itself a modification of that described by Saffigna and Waring [13] are constructed of stainless steel (Figure 1); connections between steel components in the spray trap are made with silver solder. The only glass component exposed to ammonia is the distillation flask; a PTFE sleeve is inserted between the glass flask socket and the steel cone on the trap to allow for differential expansion. The steam inlet tube (which also admits the alkali as in the Bremner apparatus) and the ammonia outlet tube are both soldered to the cover of the spray trap, which is easy to open for cleaning.

The baffles on the inlet and outlet tubes (and the slots in the outlet tube) are incorporated so that the apparatus can also be used for distilling soil extracts with MgO and Devarda's alloy for $NH_4^+$ and $NO_3^-$ determinations. Without these precautions particles of MgO can pass through the spray trap into the condenser and give erroneously high blanks.

*Procedure.* Ammonia adsorbed on surfaces within the distillation apparatus from previous distillations can exchange with $NH_3$ evolved from the current sample. To avoid errors from this 'memory effect', two separate aliquots of each digest are distilled. The first is used for measurement of total N in the sample and the second for $^{15}N/^{14}N$ ratio measurement.

The first aliquot of digest (25 ml) is placed in a 250 ml round-bottomed flask, the flask connected to the spray trap, and 20 ml 10*M* NaOH added down the steam inlet tube. Steam is passed through until 25 ml of distillate has collected in a polypropylene beaker containing 5 ml 2% boric acid; the distillate is titrated against 0.025*M* $H_2SO_4$ to pH 4.70, using a Radiometer Autotitrator.

The second aliquot of digest is placed in the same distillation flask that was used for the first (after being washed with distilled water) and is steam distilled through the *same* spray trap and condenser. The volume of the second aliquot is such as to contain about 1 mg N, as calculated from the titration of the first distillate; it is usually 50 ml. Excess 10*M* NaOH is added and 25 ml of distillate collected in a polypropylene beaker containing a slight excess of 0.025*M* $H_2SO_4$ (for example 1.5 ml is used for 1 mg N). This is evaporated to dryness on a water bath under a stream of $NH_3$-free air. The dry residue is dissolved in about 2 ml distilled water, transferred to a glass vial (2 dram soda glass screw neck Trident vial, supplied by FBG-Trident Ltd., Temple Cloud, Avon, UK) and evaporated to dryness in an oven at 80°C. The vial is capped and stored to await ratio measurement. After use the vial is discarded.

*Conversion of dried ammonium sulphate to nitrogen gas*

*Reagents.* Alkaline lithium hypobromite solution. Add 2 ml A R bromine to 60 ml 10% (w/v) $LiOH.H_2O$ solution cooled on ice. The mixture is shaken until the bromine has dissolved.

*Apparatus.* The stainless steel assembly for converting ammonium sulphate to nitrogen gas is shown in Figure 2. The reaction vessel consists of a stainless steel manifold (D) with a threaded neck and a side arm through which the liberated gas flows. A rubber septum (C) and a needle guide (B) with a hole just large enough for a 22 gauge needle are held in place by a stainless steel nut (A) taken from a $\frac{1}{4}''$ compression fitting. The septum is cut from a sheet of $\frac{1}{8}''$ thick silicone rubber septum material (type W, supplied by Pierce and

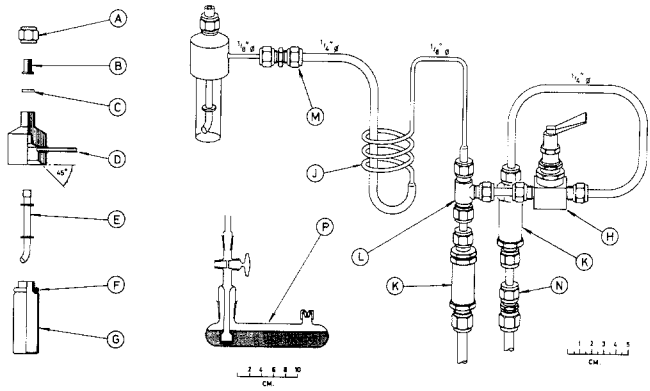


Figure 2. Assembly for production of $N_2$ gas from $(NH_4)_2SO_4$.
A Stainless steel nut from a $\frac{1}{4}$ inch compression fitting.
B Needle guide.
C Silicone rubber septum.
D Stainless steel manifold.
E Stainless steel delivery tube.
F Soft rubber O-ring.
G Glass vial containing dry $(NH_4)_2SO_4$.
H Bellows value (Nupro SS-4BKT).
J Liquid nitrogen trap.
K Micron filters (Hoke 6315 G4S), leading to mass spectrometer sample inlet (L.H.) and backing line (R.H.).
L T-piece (Hoke 4TTT-316).
M Reducing union $\frac{1}{4}$ to $\frac{1}{8}$ inch (Hoke 4RU2-316).
N Reducing union $\frac{5}{16}$ to $\frac{1}{4}$ inch (Hoke 5RU4-316).
P Degassing reservoir.

Warriner Ltd., Chester, UK); before use it is lightly rubbed over with silicone grease. Inside the manifold is a threaded stainless steel delivery tube (E), having two baffles to prevent splashes of lithium hypobromite entering the gas outlet during the reaction. The glass vial (G) is held in place by atmospheric pressure, a tightly fitting soft rubber O-ring (F) on the neck of the vial giving a vaccuum-tight seal, as in the apparatus of Ross and Martin [12]. The gas passes through a liquid nitrogen trap (J) to a bellows valve (H) which opens the line to the backing pump of the mass spectrometer. It is important that the trap J is constructed as in Figure 2; if made entirely of $\frac{1}{8}''$ diameter tubing it usually becomes blocked with ice before release of $N_2$ from a sample is complete. The whole conversion assembly was built on to the sample inlet side of a VG Micromass 602D double collector mass spectrometer.

The needle tip of the syringe used for injection (Hamilton fixed needle syringe, type 1002 N, 2.5 ml) is ground so that it is rounded. It passes through a hole in the septum previously cut with a sharp, solid needle (*not* a syringe needle). By using a lightly greased pierced septum and an accurately

fitting needle guide in this way, the blunt syringe needle always passes through the same hole in the septum: this is essential for leak-free operation of the apparatus.

Dissolved nitrogen in the lithium hypobromite is displaced by helium in the apparatus (P) shown in Figure 2. Helium gas is bubbled through the solution at about 40 ml $min^{-1}$, starting about one hour before use. Portions of lithium hypobromite are removed by the blunt syringe via the needle guide.

*Procedure.* The sample vial is initially held to the conversion assembly by hand and the preparation line evacuated using the mass spectrometer backing pump. When the line is evacuated, as seen on the Pirani gauge, the sample side of the mass spectrometer is opened. The mass spectrometer is focussed on the peak at mass 28, so that this peak can be used as a leak detector. When the value on the ratio integrator is small and there is no rise in the 28 peak, the vacuum is satisfactory. The cooling coil (J) is now immersed in liquid nitrogen and the mass spectrometer inlet taps and the bellows valve (H) are closed. Nitrogen gas is liberated from the dry sample by injecting 1 ml of degassed lithium hypobromite through the septum.

The sample inlet tap on the mass spectrometer is then opened for a few seconds to allow nitrogen to pass from the preparation line through the sample inlet section of the mass spectrometer to the variable volume reservoir. The tap to the analyser section of the mass spectrometer is then opened and the major beam current reading for mass 28 adjusted (using the variable volume reservoir) to be approximately that of the reference nitrogen sample, previously admitted via the reference gas inlet. The isotope ratio analysis is now run.

While the programmed ratio analysis is proceeding, the sample vial is removed, delivery tube baffles and manifold washed with a jet of distilled water, dried with a tissue and the cold trap warmed in a beaker of hot water. A clean dry vial is inserted into the manifold and the preparation line evacuated with the backing pump, thus removing any nitrous oxide, ammonia and water vapour condensed in the cold trap. After pumping out the preparation line, the next sample vial is placed in position for analysis. Twelve samples can be analysed per hour.

## Results

### *Recovery of ammonium- and nitrate-N by the salicylic acid modification of the Kjeldahl method*

Recovery of ammonium-N and nitrate-N by the salicylic acid modification of the Kjeldahl digestion procedure was measured in the presence of either wheat straw or soil (Batcombe series, silty clay loam, containing 28% clay, from the Broadbalk Continuous Wheat Experiment at Rothamsted).

Table 1. Recovery of ammonium- and nitrate-N by the salicylic acid modification of the Kjeldahl digestion method

| Contents of digestion tube[a] | Total N content of digest, mg | Recovery of added $NH_4$-N or $NO_3$-N, % |
|---|---|---|
| Straw | 6.44 | – |
| Straw + $(NH_4)_2SO_4$ | 11.39 | 99.0 |
| Straw + $KNO_3$ | 11.40 | 99.2 |
| Soil | 5.54 | – |
| Soil + $(NH_4)_2SO_4$ | 10.59 | 101.0 |
| Soil + $KNO_3$ | 10.60 | 101.2 |

[a] $(NH_4)_2SO_4$ and $KNO_3$ both contained 5.00 mg N

Solutions containing 5 mg N, as either $(NH_4)_2SO_4$ or $KNO_3$, were pipetted into triplicate digestion tubes and dried at 80 °C. Ground soil or plant material was added, followed by the normal digestion procedure, distillation and titration. The results were compared with those from digestions of either soil, straw, $(NH_4)_2SO_4$ or $KNO_3$ alone. Recovery of both $NH_4$-N and $NO_3$-N was virtually complete in the presence of either soil or straw (Table 1).

In this method the solution of salicylic acid in concentrated sulphuric acid is added to the *dry* soil as the reduction of nitrate is incomplete in the presence of water. However the Kjeldahl digestion procedure can give low results with certain soils unless they are wetted with water before addition of concentrated sulphuric acid [1]. This effect is most serious with coarsely ground soils [9] and was not observed in our work, using disc milled soil, with more than 95% passing a 60 mesh sieve. Thus a topsoil, selected to have a low nitrate-N content (from plot 06 of the Broadbalk Continuous Wheat Experiment at Rothamsted; 0–23 cm), gave a nitrogen content of 0.1215% ± 0.00032 (S.E.) when wetted before addition of the salicylic/sulphuric acid and 0.1208% ± 0.00016 (S.E) when the acid mixture was added to dry soil. A subsoil (also from Broadbalk plot 06, 50–70 cm) gave a value of 0.0531% ± 0.00032 (S.E.) when wetted and 0.0535% ± 0.00028 (S.E.) when the acid mixture was added to dry soil.

*Prevention of cross contamination in the steam distillation apparatus*

A small fraction of the ammonia released from the solution in the distillation flask is adsorbed on surfaces within the distillation apparatus. If the adsorption sites are already occupied by ammonia molecules from previous samples, the freshly released ammonia will undergo partial or complete exchange with the previously adsorbed ammonia. If the previously adsorbed ammonia has a different $^{15}N$ enrichment from the current sample the enrichment measured in the distillate will be incorrect. This so-called "memory effect" is less with a metal trap and condenser than with glass apparatus [10, 13] but is still sufficient to cause unacceptable errors in precise work.
The problem can be overcome by displacing all adsorbed ammonia before

Table 2. "Memory effect" in steam distillation apparatus

| Sample[a] | Atom % excess $^{15}N$ measured in distillate[b] |
|---|---|
| Unlabelled $(NH_4)_2SO_4$ before distilling enriched solution | 0.0011 |
| $^{15}N$-labelled $(NH_4)_2SO_4$ | 4.7559 |
| Unlabelled $(NH_4)_2SO_4$ after distilling enriched solution: | |
| first aliquot | 0.0030 |
| second aliquot | 0.0012 |
| third aliquot | 0.0011 |

[a]Each sample contains 1 mg N. Same flask and distillation apparatus used throughout.
[b]Mean of results from two separate experiments using different distillation apparatus.

each sample is distilled. This can be achieved by distilling ethanol between samples [2] or by passing steam through the apparatus with the condenser cooling water turned off [13]. An alternative procedure is to distil two separate aliquots from each sample, as described above. Because the distillate from the first aliquot is used for total N measurement only, any alteration in its $^{15}N$ enrichment causes no error. Adsorbed ammonia from the first aliquot will exchange during distillation of the second, but this will not cause error as it will have almost the same $^{15}N$ enrichment as that being evolved. Table 2 shows that this double distillation procedure is effective in eliminating the "memory effect". The atom percent excess $^{15}N$ measured in the distillate from unenriched ammonium sulphate, distilled immediately after a solution containing 4.756 atom percent excess $^{15}N$, was 3 times that measured from an aliquot distilled before the enriched solution. However, the enrichment in the distillate of the *second* aliquot of unenriched solution, distilled through the same apparatus, was indistinguishable from that in the original distillate.

The results in Table 2 indicate that ammonia adsorbed during one distillation is almost completely displaced during the next. This being so, it can be calculated that 0.4 $\mu$g of ammonia N is held on surfaces within the distillation apparatus. In an earlier apparatus, in which a glass spray trap was used, 2.8 $\mu$g N was held but even with the glass trap it was found that the double distillation procedure was as effective as an ethanol distillation in eliminating the "memory effect".

An additional advantage of the double distillation procedure is that it is not possible for ammonia adsorbed on the glass electrode used for titration to cause a further memory effect [13]. With the proposed procedure the second distillate is not titrated; the volume of $H_2SO_4$ necessary to give a suitable excess is calculated from the result of the titration of the first distillate.

*Preparation of nitrogen gas for $^{15}N$ analysis*

The main difference between the new preparation line and those currently used [3, 11, 12] is that lithium hypobromite solution is degassed in a

separate reservoir (P) and is injected via a septum into the sample vial using a syringe. The all stainless steel apparatus is more robust than the other systems and its operation is rapid and simple.

Previous workers have found that it is necessary to immerse the sample vial in liquid nitrogen or an acetone/dry ice mixture before addition of hypobromite solution if cross contamination between samples is to be avoided [11, 12]. In our procedure it is not necessary to freeze the sample either before or after addition of hypobromite. To demonstrate this, portions of unlabelled $(NH_4)_2SO_4$, each containing 1 mg N, were treated with lithium hypobromite solution; the measured atom percent excess was 0.0009 ± 0.00011 (S.E.). Three samples of $(NH_4)_2SO_4$ containing 1 atom percent excess $^{15}N$ were then processed, followed by a further unlabelled sample. The atom percent excess measured in this final sample was 0.0008, indicating that no detectable cross contamination had occurred.

The total volume of the preparation line is 22 ml, including 11 ml for the sample vial. The system is designed to handle samples containing 1 mg N, but 0.2–2 mg samples can be used. $^{15}N/^{14}N$ ratios can be measured on samples containing only 25 $\mu$g N, but with lower accuracy and precision.

A possible source of error with this system is contamination of sample $N_2$ by atmosphere $N_2$, due to:

(a) incomplete displacement of $N_2$ by He in the lithium hypobromite solution;

(b) absorption of atmospheric $N_2$ by hypobromite in the syringe during transfer from the degassing apparatus to the preparation line;

(c) leakage through the septum, particularly while the syringe is being inserted or removed.

To test for such contamination, 1 ml of hypobromite solution was injected into an empty vial, with the preparation line open to the mass spectrometer. The whole system had previously been pumped down, the trap cooled with liquid nitrogen and the mass spectrometer focussed on mass 28. The background ion current increased from 21 to 46 (mean of 3 measurements) when hypobromite was injected. Under normal operating conditions, 1 mg N gives an ion current at mass 28 of about 80, 000 so the input of atmospheric $N_2$ on injection of hypobromite was 0.3 $\mu$g N, equivalent to 0.03% of a normal sample.

The results of a different test for atmospheric contamination are shown in Table 3. Six replicate portions of enriched $(NH_4)_2SO_4$, containing either 1 mg or 0.5 mg N, were analysed. If the same amount of atmospheric $N_2$ enters the preparation line each time a sample is treated with LiOBr, the atom % excess $^{15}N$ would be less if measured on a 0.5 mg sample than on a 1 mg sample. The scatter of the measurements was slightly greater with 0.5 mg N than with 1 mg (Table 3), presumably because the smaller samples produced smaller ion currents so that the $^{15}N/^{14}N$ ratio was measured under sub-optimal conditions. However, the mean atom percent excess in the 0.5 mg

Table 3. Effect of sample size on measured $^{15}N$ content using the proposed preparation line

| Sample size | Measured atom % excess $^{15}N$ |
|---|---|
| 1.0 mg N | 0.9695 |
| | 0.9710 |
| | 0.9712 |
| | 0.9704 |
| | 0.9707 |
| | 0.9706 |
| | Mean 0.9706 ± 0.00024 (SE) |
| 0.5 mg N | 0.9701 |
| | 0.9702 |
| | 0.9713 |
| | 0.9693 |
| | 0.9711 |
| | 0.9700 |
| | Mean 0.9703 ± 0.00030 (SE) |

samples did not differ significantly from that measured in the 1 mg samples, indicating that the accretion of atmospheric $N_2$ in the preparation line was too small to have a measurable effect on the results. As it is so happens, the difference in the two mean values shown in Table 3 (0.0003 atom % $^{15}N$) is exactly what would be found if the input of atmospheric N was 0.3 $\mu$g per measurement, as calculated above.

*Measurement of atom % excess $^{15}N$ in $N_2$ gas*

The $^{15}N/^{14}N$ ratio in the sample gas was compared with that of a reference gas (White Spot Nitrogen supplied by the British Oxygen Company). The atom % $^{15}N$ of this reference gas was 0.3654 (= y), measured against atmospheric $N_2$ (taken as containing 0.3663 atom % $^{15}N$; for a justification of the use of atmospheric $N_2$ as standard see Mariotti [8]. The mass spectrometer is calibrated twice a day by admitting reference gas to both sample and reference gas sides of the instrument and measuring the ion current ratio for masses 29/28. The value thus obtained (R′, mean of 6 measurements) is substituted in the equation

$$\text{atom \% } ^{15}\text{N in reference gas } (= x) = \frac{100\,R'}{2 + R'}$$

The machine factor (u) is given by

$$u = \frac{y}{x}$$

The mass spectrometer takes 6 measurements of the sample ion current ratio, interleaved with 6 measurements on the reference gas. The atom % excess $^{15}N$ in the sample is calculated from the mean ratio for the sample gas ($R_S$) and for the reference gas ($R_R$) by the formula

$$\text{Atom \% excess } {}^{15}\text{N in sample gas} = \frac{200\ u\ (R_S - R_R)}{(2 + R_S)(2 + R_R)}$$

using a specially written programme for the HP 97S calculator fitted to the mass spectrometer.

*Calculation of results*

The amount of N derived from labelled fertilizer in a crop or soil sample is calculated from the expression:

$$F = T\left(\frac{p - q}{f}\right)\left(\frac{t_b}{t_s - t_b} + 1\right)\left(\frac{M_1}{M_2}\right)$$

Where:

F = Weight of N derived from labelled fertilizer in crop or soil sample;

T = Total weight of N sample (T and F are expressed in the same units e.g. $\mu g\ N\ g^{-1}$ sample, or $kg\ N\ ha^{-1}$) calculated using an atomic weight of 14 for nitrogen. In calculating T from the titration value $t_s$ the reagent blank $t_b$ is the first subtracted. True total N is given by $T\ \frac{M_3}{M_2}$.

p = atom percent excess $^{15}N$ in labelled sample of crop or soil;

q = atom percent excess $^{15}N$ in control sample of crop or soil that did not receive labelled fertilizer;

f = atom percent excess $^{15}N$ in labelled fertilizer as added;

$t_s$ = volume of $H_2SO_4$ required for titration of distillate from an aliquot of the Kjeldahl digest;

$t_b$ = volume of $H_2SO_4$ required for titration of distillate from the same aliquot of the blank Kjeldahl digest;

$M_1$ = the (true) average atomic weight of N in the labelled fertilizer

$$= \frac{(\text{atom percent } {}^{15}\text{N} \times 15) + (\text{atom percent } {}^{14}\text{N} \times 14.003)}{100}$$

$M_2$ = the (erroneous) atomic weight (= 14) of N used in calculating the total N content of the sample;

$M_3$ = the (true) average atomic weight of N in the sample, calculated as for $M_1$.

This equation differs from that given by Hauck and Bremner [6] in the two correction factors. The factor $\left(\frac{t_b}{t_s - t_b} + 1\right)$ corrects for N in the Kjeldahl reagents, assuming that the blank titre ($t_b$) is wholly due to reagent

N at natural abundance. The factor $\frac{M_1}{M_2}$ corrects for the atomic weight (14) used in calculating the total N content of the crop or soil sample; it is usually insignificant.

*Analytical precision*

Table 4 shows the results of 10 analyses, done over a period of 5 weeks on portions of a bulk sample of $^{15}N$ labelled grass. For total N, the coefficient of variation (CV) was 0.78%; for atom % excess $^{15}N$ was 0.12%.
Table 5 shows how sampling errors are distributed in a field experiment. Winter wheat was given labelled fertilizer N at four different rates, each rate being applied to three replicate plots [7]. Samples of grain, straw and soil were collected from each plot at harvest (one of each per replicate plot) and analysed in duplicate. For replicate analyses of total N on a single sample of grain the CV was 1.1%, for straw 0.9%, and for soil 1.1, giving a mean CV of 1.0% for all the results in Tables 4 and 5. The CV of replicate analyses of a single sample of grain for atom % excess $^{15}N$ was 0.15% and for straw 0.4%, giving a mean CV of 0.2% for all the *plant* samples in Tables 4 and 5. The CV for atom % excess $^{15}N$ in soil was higher (1.2%), presumably because the measured abundances were so close to natural.

Although not the concern of the present paper, Table 5 also shows that between-plot CVs were very much larger than between-sample CVs, i.e. field heterogeneity contributed much more to overall sampling error than did analytical errors in the laboratory. The data in Table 5 are typical of those we

Table 4. Analyses of a standard grass sample

| Sample No.[a] | %N[b] | Atom % excess $^{15}N$[c] |
|---|---|---|
| 1 | 1.417 | 0.4152 |
| 2 | 1.403 | 0.4153 |
| 3 | 1.416 | 0.4150 |
| 4 | 1.423 | 0.4158 |
| 5 | 1.396 | 0.4158 |
| 6 | 1.386 | 0.4154 |
| 7 | 1.403 | 0.4149 |
| 8 | 1.411 | 0.4159 |
| 9 | 1.413 | 0.4163 |
| 10 | 1.405 | 0.4163 |
| | 1.407 ± 0.0110(SD) | 0.4156 ± 0.00050(SD) |

[a]Two samples weighed from a bulk sample of Tema-milled grass were each analysed for % N and atom % excess $^{15}N$ during the course of a week's work. Samples 1 and 2 were analysed during the first week, 3 and 4 during the succeeding week and so on.
[b]Expressed on an oven-dry (24 h at 80 °C) basis.
[c]Taking natural abundance as 0.3663 atom %.

Table 5. Between-plot and between-sample errors

| Plot No.[a] | Application of labelled fertilizer N, kg ha$^{-1}$ | Grain | | | Straw | | | Soil | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Value | ± SD (between plots[c]) | ± SD (between samples[d]) | Value | ± SD (between plots[c]) | ± SD (between samples[d]) | Value | ± SD (between plots[c]) | ± SD (between samples[d]) |
| | | | | | | %N | | | | |
| 06 | 49.3 | 1.626 | 0.032 | 0.016 | 0.268 | 0.028 | 0.004 | 0.1174 | 0.0046 | 0.0021 |
| 07 | 97.5 | 1.805 | 0.065 | 0.019 | 0.348 | 0.015 | 0.002 | 0.1273 | 0.0051 | 0.0015 |
| 08 | 147.4 | 1.992 | 0.018 | 0.027 | 0.493 | 0.046 | 0.005 | 0.1292 | 0.0023 | 0.0003 |
| 09 | 195.8 | 2.249 | 0.074 | 0.018 | 0.690 | 0.064 | 0.005 | 0.1263 | 0.0057 | 0.0012 |
| All plot mean | – | 1.918 | 0.053(2.7)[e] | 0.020(1.1) | 0.449 | 0.042(9.4) | 0.004(0.9) | 0.1251 | 0.0046(3.7) | 0.0014(1.1) |
| | | | | | Atom % excess $^{15}$N | | | | | |
| 06 | 49.3 | 1.566 | 0.161 | 0.004 | 1.642 | 0.123 | 0.009 | 0.0252 | 0.0019 | 0.0002 |
| 07 | 97.5 | 2.102 | 0.225 | 0.002 | 2.231 | 0.173 | 0.015 | 0.0351 | 0.0011 | 0.0003 |
| 08 | 147.4 | 2.411 | 0.098 | 0.001 | 2.534 | 0.112 | 0.006 | 0.0446 | 0.0049 | 0.0006 |
| 09 | 195.8 | 2.848[f] | 0.011 | 0.005 | 2.924 | 0.045 | 0.006 | 0.0481 | 0.0034 | 0.0006 |
| All plot mean | – | 2.231 | 0.147(6.6) | 0.0033(0.15) | 2.333 | 0.122(5.2) | 0.0099(0.4) | 0.0382 | 0.0032(8.3) | 0.00046(1.2) |

[a]From the 1981 $^{15}$N experiment on the Broadbalk Continuous Wheat Experiment at Rothamsted (see Jenkinson, Johnston, Powlson, Pruden and Williams, 1981).
[b]Applied as $NH_4NO_3$ at 5 atom % excess $^{15}$N.
[c]Three replicate plots per treatment; 2 degrees of freedom (DF) for between-plot standard deviations (SD).
[d]One sample per plot, analysed in duplicate; 3 DF for between-sample SD's.
[e]Eight DF for between-plot SD's 12 for between-sample SD's; coefficients of variation in parentheses.
[f]One analysis rejected.

have obtained by the application of the proposed methods to routine field work with $^{15}N$; they were taken from a very much larger collection of data, accumulated over several years and are in no way exceptional.

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