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Review and simplification of calculations in ^{15}N tracer studies

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Abstract

Calculations in nitrogen (N) balance research using ^{15}N involve several steps that require care to avoid errors. The objective of this paper is to provide examples of these calculations using established procedures and to present shortened alternative calculations that give the same result. The calculations examined include determination of the amount of N to apply, determination of the atom % ^{15}N abundance needed in the labeled fertilizer, preparation of the labeled fertilizer, and calculation of the fertilizer N recovered. Calculations needed in the preparation of the labeled fertilizer using established procedures include the determination of the mean atomic weight of the enriched source from which the labeled fertilizer is prepared. This determination is not needed in the shortened alternative calculations, because the procedure places the calculations on a mole basis rather than a mass basis.

Introduction

The use of ^{15}N-labeled fertilizer in N tracer studies requires several calculations in the preparation stage of the experiment and in determining a final N balance. Several equations for these calculations were given by Hauck and Bremner [1976]. However, step by step examples of calculations were not provided. The purposes of this paper are to 1) provide example calculations using established procedures and 2) present shortened alternative calculations that give the same result.

Before proceeding with the description of the procedures, we shall define two important terms used in the paper: atom % ^{15}N abundance and atom % ^{15}N excess. Atom % ^{15}N abundance is the percentage of nitrogen atoms with a mass of 15 in a sample. For example, atmospheric N has approximately 0.366 atom % ^{15}N abundance. The ^{15}N content of a sample can also be expressed as the excess atom % ^{15}N over that present in atmospheric N (i.e. atom % ^{15}N abundance − 0.366). This value is called the atom % ^{15}N excess of the sample.

The calculations needed in a ^{15}N-labeled fertilizer study can be divided as follows:

1. determination of the amount of N to apply;
2. determination of the atom % ^{15}N abundance needed in the labeled fertilizer;
3. preparation of the labeled fertilizer; and
4. calculation of fertilizer N recovered.

Typical procedure

In a typical procedure, the steps indicated above are performed in the following manner.

1. Determination of the amount of N to apply

Assume that we will use a microplot 0.36 m^2 in area and that we would like to apply the equivalent of 200 kg N/ha. The amount to apply would be

$$200\,\text{kg}\,\frac{\text{N}}{\text{ha}} \times \frac{1000\,\text{g}}{\text{kg}} \times \frac{1\,\text{ha}}{10{,}000\,\text{m}^2} \times 0.36\,\text{m}^2$$

$$= 7.2000\,\mathrm{g\ N}$$

Since ^{15}N-labeled fertilizer has a larger atomic weight than non-labeled fertilizer, we need to express the amount to apply in moles of N. To do this, we need to use the mean atomic weight of naturally occurring N, which is 14.0067.

$$7.2000\,\mathrm{g}\,\frac{\mathrm{N}}{\mathrm{microplot}} \times \frac{1\,\mathrm{mol}}{14.0067\,\mathrm{g}}$$

$$= 0.5140397 \text{ moles of N}$$

In order to reduce the effect of rounding-off errors, we shall carry several significant digits in our calculations.

2. Determination of the atom % ^{15}N abundance needed in the labeled fertilizer

After ^{15}N-enriched fertilizer is mixed with the soil, it may be partitioned into various pools such as organic N, the plant, or N gases (N_2, N_2O, or NH_3). Incorporation into the various soil N pools results in dilution of the enriched fertilizer, with the amount of dilution being determined by the amount of ^{15}N moving into a pool and the total amount of N in that pool. The pool of N that will result in the greatest dilution of ^{15}N will determine the atom % ^{15}N needed in the fertilizer.

In order to determine the atom % ^{15}N abundance needed in the fertilizer, the following equation can be used:

$$\text{Final Pool At\% ab.} = (\text{Fert At\% ab.} \times \text{Prop Fert}) + (\text{Initial Pool At\% ab.} \times \text{Prop Pool.}) \qquad (1)$$

where

Final Pool At % ab. = minimum acceptable atom % ^{15}N abundance at the completion of the experiment in the pool causing the greatest dilution. This value will be determined by the sensitivity of the mass spectrometer, as described later.

Fert At % ab. = atom % ^{15}N abundance of the fertilizer N.

Initial Pool At % ab. = initial atom % ^{15}N abundance in the pool causing the greatest dilution (diluting pool). This value is typically 0.366 atom % ^{15}N in plants, whereas it usually varies between 0.368 and 0.370 in soils.

Prop Fert = proportion of all nitrogen atoms in the final mixture (soil + fertilizer) from the fertilizer N.

Prop Pool = Proportion of all nitrogen atoms in the final mixture (soil + fertilizer) from the soil N pool of interest.

Since we wish to solve for Fert At % ab., the equation can be rearranged to give

$$\text{Fert At\% ab.} = \frac{\text{Final Pool At\% ab.} - (\text{Initial Pool At\% ab.} \times \text{Prop Pool})}{\text{Prop Fert.}} \qquad (2)$$

In order to use this equation, the sensitivity of the mass spectrometer must be considered. Suppose our mass spectrometer can determine the natural abundance of 0.366 atom percent ^{15}N with an accuracy of ±0.001 atom percent. If we are willing to accept a measurement accuracy of ±10%, the lowest value of any soil or plant sample assayed would need to be $\geqslant 0.376$ atom percent ^{15}N, since

$$\frac{0.001}{0.376 - 0.366} \times 100 = 10\%$$

Therefore, we would assign a value of 0.376 for Final Pool At % ab. in equation [2].

In order to calculate the required atom % ^{15}N abundance of the fertilizer (Fert At % ab.), we also need to select the minimum detectable proportion of the N atoms in the assayed material coming from the fertilizer (Prop Fert) that we consider acceptable. The proportion selected will depend on the size of the diluting pool. For example, if we wish to detect the incorporation of 0.3 mmoles of fertilizer N per kg of soil in a soil organic N pool of 99.7 mmoles of N per kg of soil, the proportion from fertilizer selected would be

$$\text{Prop Fert} = \frac{0.3}{99.7 + 0.3} = 0.003.$$

Then, using equation (2),

$$\text{Fert At\% ab.} = \frac{0.376 - 0.366\ (0.997)}{0.003} =$$

3.699 atom % ^{15}N abundance.

If the diluting pool is smaller, then the required atom percent needed would be smaller also. For example, the pool size of soil NO_3^--N might be 2.7 mmoles of N per kg of soil. If we wish to detect the inclusion of 0.3 mmoles of fertilizer NO_3^--N per kg of soil into this pool, the proportion selected would be

$$\text{Prop Fert} = \frac{0.3}{2.7 + 0.3} = 0.10$$

For this latter case, the atom % ^{15}N abundance needed in our fertilizer source calculated with equation (2) would be

$$\text{Fert At\% ab.} =$$

$$\frac{0.376 - 0.366\ (0.9)}{0.1} =$$

0.466 atom % ^{15}N abundance.

3. *Preparation of the labeled fertilizer*

a. Proportions of N atoms needed from labeled and unlabeled sources. Very often, it is necessary to prepare the labeled fertilizer from a source that is highly enriched in ^{15}N. Typically, this is done by mixing the highly ^{15}N-enriched source with another source containing ^{15}N at natural abundance levels (diluting source). Since the diluting source usually has 0.366 atom % ^{15}N abundance, it is easier to perform calculations using atom % ^{15}N excess values rather than atom % ^{15}N abundance levels. For illustration purposes, let's assume that we have a source of fertilizer containing 50 atom % ^{15}N abundance and that we want to prepare labeled fertilizer containing 5 atom % ^{15}N abundance as determined from step 2. The following equation can be used to determine the atom % ^{15}N excess of a mixture of two sources with different ^{15}N contents.

$$A_1 = A_0 E$$

where

A_1 = resulting atom % ^{15}N excess in the mixture.
A_0 = atom % ^{15}N excess of the enriched source.
E = proportion of all nitrogen atoms in the final mixture from the enriched N source (thus, 1 − E would be the proportion of nitrogen atoms coming from the diluting source).

Knowing A_1 and A_0, we can find E.

$$E = \frac{A_1}{A_0}$$

In our case, $A_1 = (5 - 0.366)$, $A_0 = (50 - 0.366)$.

$$E = \frac{5 - 0.366}{50 - 0.366} = 0.09336$$

$$1 - E = 1 - 0.09336 = 0.90664$$

b. Moles needed from each source. Thus, of the total moles of N needed in the mixture, 90.664% have to come from the diluting source and 9.336% from the enriched source. We have calculated before that we needed 0.5140397 moles of N. Thus, the moles needed of each source are as follows:

0.5140397 moles N (mixture) × 0.90664 (from 0.366 at % ^{15}N ab.)

= 0.4660489 moles from 0.366 at % ^{15}N ab.

0.5140397 moles N (mixture) × 0.09336 (from 50 at % ^{15}N ab.)

= 0.0479907 moles from 50 at % ^{15}N ab.

c. Weight of N needed from each source. To calculate the weight of N needed from each source, it is necessary to know the mean atomic weight (Mn) for N in each source. This value can be calculated from:

$$Mn = 0.01\ (\text{atom \% }^{15}\text{N ab.} \times 15.000 + \text{atom \% }^{14}\text{N ab.} \times 14.003)$$

The values of 15.000 and 14.003 represent the atomic weights of ^{15}N and ^{14}N isotopes of nitrogen, respectively.

Mn (diluting) = 0.01 (0.366 × 15.000 + 99.634 × 14.003) = 14.0067

Mn (enriched) = 0.01 (50 × 15.000 + 50 × 14.003) = 14.5015

0.4660489 moles of 0.366 at % ^{15}N ab. × 14.0067 g/mol = 6.527807 g N from source 0.366 at % ^{15}N ab.

0.0479907 moles of 50 at % ^{15}N ab. × 14.5015 g/mol = 0.695937 g N from source with 50 at % ^{15}N ab.

So far, we have calculated the weights of N needed (from enriched and diluting sources) to obtain a certain atom % ^{15}N abundance in the fertilizer to be applied. Since the sources used are usually not 100% N, we need to calculate the actual weights of each N source needed to provide the weights of N required. For that purpose, we need to know the N concentration of each N source (g N/g source).

d. N concentration of each source. Assume that the source we are using for enriching and diluting fertilizer is urea and that we digest 200 mg of each source for analysis of percent N. Assume also that we dilute the digest to 1000 mL and that we use 10 mL for steam distillation. The amount of titer (net) will be different for each source; let's suppose that we titrated with 0.005 N H_2SO_4 to reach the endpoint. If we used 13.32 mL to titrate the diluting source sample, the N concentration in this source would be

$$\frac{13.32\,\text{mL} \times 0.005\,\text{N} \times 14.0067 \times 1000\,\text{mL}}{200\,\text{mg urea} \times 10\,\text{mL}} = 0.46642\,\text{g N/g source} \qquad (3)$$

If we used 13.10 mL to titrate the enriched source sample, the N concentration in this source would be

$$\frac{13.10\,\text{mL} \times 0.005\,\text{N} \times 14.5015 \times 1000\,\text{mL}}{200\,\text{mg urea} \times 10\,\text{mL}} = 0.47492\,\text{g N/g source}$$

e. Weight of each source needed. Amount of diluting source to apply:

$$6.527807\,\text{g N from } 0.366 \text{ at \% } ^{15}\text{N ab.} \times \frac{1\,\text{g source}}{0.46642\,\text{gN}} = 13.996\,\text{g}$$

Amount of enriched source to apply:

$$0.695937\,\text{g N } 50 \text{ at \% } ^{15}\text{N ab.} \times \frac{1\,\text{g}}{0.47492\,\text{gN}} = 1.465\,\text{g}$$

4. Calculation of fertilizer N recovered

Assume that at harvest, we measure 1 mol of N in the plants of each microplot and that the ^{15}N analysis indicates 0.8 atom % ^{15}N abundance. The following equation presented by Hauck and Bremner (1976) can be used to calculate percent recovery of nitrogen fertilizer.

$$\%\text{ N recovered} = 100 \times \frac{p(c - b)}{f(a - b)}$$

where

p = moles of N in the plant material
f = moles of N in the fertilizer
c = atom % ^{15}N abundance in the plant
a = atom % ^{15}N abundance of the fertilizer
b = atom % ^{15}N abundance of plants grown in unfertilized soil (assume 0.366 for this example)

$$\%\text{ N recovered} = 100 \times \frac{1\,\text{mol}\,(0.8 - 0.366)}{0.5140397\,\text{mol}\,(5 - 0.366)} = 18.22\%$$

While it is probably valid to use a background of 0.366 atom % ^{15}N abundance for plants, it is wise to experimentally determine the background for soil, because these values usually vary between 0.368 and 0.370 atom % ^{15}N abundance.

The percent N recovered also can be calculated using only the values of 'f' and 'a' corresponding to the enriched source. As calculated before, we used 0.0479907 moles of 50 at % ^{15}N abundance.

$$\%\text{ N recovered from fertilizer} = 100 \times \frac{1\,\text{mol}\,(0.8 - 0.366)}{0.0479907\,(50 - 0.366)} = 18.22\%$$

In this formula, only the moles of labeled fertilizer N and its associated atom % ^{15}N abundance are used to calculate any part of or the entire N balance. It would be assumed that the untagged fertilizer had the same fate as the tagged fertilizer applied to the microplot. If one applies both in the same physical from, this assumption will be correct.

Alternative procedure

In the alternative procedure that we propose, it is not necessary to calculate the mean atomic weight of the enriched source because the procedure places the calculations on a mole basis rather than a mass basis. That the correction for atomic weight is not needed can be shown with the following example. The alternative procedure is the same as the typical procedure through step 3b, and step 3c is omitted. From then on, the following steps apply:

3d′. N concentration of each N source (mol N/g source)

Since we shall not use weights but moles in our calculations, we need to know the N concentration of each N source in moles N/g source. This can be calculated with equation (3) if the atomic weight of N is omitted, i.e., 14.00667. The unlabeled fertilizer N concentration is 0.03330 moles N/g of fertilizer, whereas the labeled fertilizer concentration is 0.03275 moles N/g fertilizer.

3e′. Weight of each source needed

in step 3b, we found the moles of N needed of each source. The weight of enriched fertilizer needed would be as follows:

$$0.0479907 \text{ moles } 50 \text{ At\% } {}^{15}\text{N ab.} \div \frac{0.03275 \text{ moles N}}{\text{g enrich. fert.}} = 1.465\,\text{g enrich. fert.}$$

The weight of diluting fertilizer can likewise be calculated as follows:

$$0.4660489 \text{ moles } 0.366 \text{ At\% } {}^{15}\text{N ab.} \div \frac{0.0330 \text{ moles N}}{\text{g diluting fert.}} = 13.996\,\text{g diluting fert.}$$

The calculation of the fertilizer N recovered with the alternative procedure is the same as for the typical procedure described in section 4. Calculations may be done using the atom % ^{15}N abundance of the fertilizer mixture or that of the enriched source.

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References

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