A Heuristic Model for the Calculation of Dinitrogen and Nitrous Oxide Flux from Nitrogen-15-Labeled Soil

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ABSTRACT

Very sensitive measurements of N₂ and N₂O flux from soil are possible when gas evolved from ¹⁵N-labeled soil is analyzed by isotope ratio mass spectrometry. This approach is useful for studying the fate of nitrogen fertilizer and for studying soil microbial processes contributing to the atmospheric increase of nitrous oxide, a radiatively active trace gas that can contribute to global warming and ozone depletion. Most systems of equations that relate isotopic analysis to gas flux are sufficiently complex that certain limitations and potentials of the ¹⁵N approach may be overlooked. We describe a graphical representation of labeled N-gas flux that illustrates the equations and encourages critical thinking regarding the implementation of related experiments. This model is used to interpret underestimation that occurs if flux derives from multiple pools of differing enrichment. A statistical derivation is presented for a previously published simulation of underestimation due to multiple pools. The same equations are applied to field data to explore whether temporal variation in soil nitrate enrichment is likely to cause significant underestimation. Two sampling strategies are proposed that may eliminate the assumption of pool uniformity, thereby eliminating a potential source of underestimation.

Dinitrogen and nitrous oxide are alternative end products of microbial denitrification. Quantifying their flux from soil can help explain fertilizer losses from agricultural systems (Mosier et al., 1986; Eichner, 1990; Weier et al., 1993) as well as the atmospheric buildup of N₂O — an important greenhouse gas in the troposphere and ozone-destuctive catalyst in the stratosphere (Bouwman, 1990; IPCC, 1996). However, field studies of denitrification have been hampered by the insensitivity of standard instrumentation to N₂ increases, e.g., under soil covers (Mosier and Klemdtsston, 1994).

Mass spectrometric analysis of gas from ¹⁵N-labeled soil is a sensitive method for quantifying the flux of N₂, as well as N₂O, because of the low natural abundance of ¹⁵N. Isotopic data for headspace gases can also estimate the enrichment of the source N pool, can help identify the source of N for N₂O, and can be used to check assumptions of the flux method (e.g., Stevens et al., 1997; Arah, 1997). Recent advances in spectrometer sensitivity and affordability have generated new interest in using ¹⁵N for soil biogeochemical investigations. Given the complexity of the system of equations normally used for interpreting the isotope data, it is useful to have conceptual tools which make the principles of the system more intuitive.

We present here a heuristic model that illustrates the estimation of source pool enrichment and estimation of soil-derived headspace gas for N₂ or N₂O from ¹⁵N-labeled soil. A convenient graphical representation of N isotope data is identified, from which a complete set of equations is derived by geometric inference. These equations are similar in form and identical in function to those of others (Siegel et al., 1982; Mulvaney, 1984; Arah, 1992). To illustrate utility, the model is applied to the problem of underestimation that occurs when flux derives from multiple pools of differing enrichment.

Measuring Flux

Flux of N₂ produced by denitrification in a ¹⁵N-enriched soil can be measured by monitoring the increase in enrichment of headspace gas in a chamber placed over the soil. Proper analysis requires measurement of the abundance of all three molecular masses of N₂ (28, 29, 30). When atoms of ¹⁵N are distributed randomly among a sample of N₂ molecules, measurement of any two masses suffices, because the abundance of the third mass can be predicted statistically. However, a mixture of N₂ from two differently labeled sources (e.g., enriched N₂ from the soil and unenriched atmospheric N₂ in an enclosure) is not in isotopic equilibrium (Hauck et al., 1958): that is, the isotopes of N are not randomly distributed among the three molecular fractions. This means that all three masses must be measured, which has the additional advantage of providing an indirect estimate of the average enrichment of the soil N pool (Hauck and Bouldin, 1961). The estimation of enrichment is convenient because it is non-destructive and is a time-weighted mean.

Equations for the determinations of flux and source enrichment, by isotope ratio mass spectrometry, are well established (Siegel et al., 1982; Mulvaney and Boast, 1986; Mulvaney, 1984; Arah, 1992). These equations are designed to measure total flux of N₂ and assume that the gas is derived from a single, uniformly labeled pool of soil N. (In the absence of pool uniformity, flux derived from added label—e.g., fertilizer—can be estimated, but not by these equations.) The same principles apply for N₂O; for simplicity, most of this discussion is limited to N₂.

Graphical Representation

We adopt, wherever possible, the notations and definitions of Arah (1992). Briefly, the ¹⁵N atom fraction ¹⁵a of any sample of N is the total number of ¹⁵N atoms

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divided by the total number of N atoms (informally, the enrichment). The molecular fractions $^{28}x$, $^{29}x$, and $^{30}x$ are the fractions of the total number of N$_2$ molecules in a sample with masses 28, 29, and 30, respectively. A sample of N$_2$ is in isotopic equilibrium if the molecular fractions follow a binomial distribution:

$$^{28}x = (^{15}a)^2$$  \[1a\]
$$^{29}x = 2(^{15}a)^2 (^{14}a)$$  \[1b\]
$$^{30}x = (^{15}a)^2$$  \[1c\]

where

$$^{14}a = 1 - ^{15}a$$  \[1d\]

All N$_2$ (or N$_2$O) derived from uniform pools of molecules with single N atoms (e.g., nitrate) by microbial and chemical processes (e.g., denitrification) are expected to be in isotopic equilibrium, even if isotopic fractionation is significant (i.e., even if one isotope is inherently favored by the process). All mixtures of two or more equilibrium samples with different $^{15}$N atom fractions are necessarily nonequilibrium mixtures. Atmospheric N$_2$ is assumed to be in isotopic equilibrium.

Additionally, we define the isotopic character of an N$_2$ sample as the relative proportions of the three molecular fractions. We represent the isotopic character of N$_2$ by plotting $^{29}x$ versus $^{15}a$. The fact that such a plot is equivalent to a ternary plot of $^{29}x$, $^{28}x$, and $^{30}x$ (Fig. 1; Note 1) is both a mathematical convenience and an informal proof that $^{29}x$ and $^{15}a$ completely characterize N$_2$ isotopically. The isotopic character of a single sample may be expressed as the coordinate pair ($^{15}a$, $^{29}x$).

Figure 2 represents graphically the calculation of source pool enrichment and flux from a plot of isotopic character. The downward-opening parabola is called the equilibrium curve, and has the form of Eq. [1b]; it represents the isotopic characters of all possible N$_2$ samples that are in isotopic equilibrium. Whenever N atoms are paired randomly from a source of given enrichment, $^{29}N_2$ will be a quadratic function of $^{15}N$, with no $^{29}N_2$ produced when all or none of the source atoms have mass 15.

The symbol $A$ represents the isotopic character of atmospheric N$_2$ already present in a chamber headspace. $P$ represents the isotopic character of N$_2$ derived from the soil pool; since the soil pool consists of uniformly enriched mineral N, Component $P$ is initially in isotopic equilibrium. $M$ represents the isotopic character of a mixture of atmospheric and soil-derived N$_2$ in a chamber headspace (this mixture is not in isotopic equilibrium). Plotted thus, the isotopic character of any mixture is a linear interpolation between its two constituents, and its distance from either is inversely proportional to the relative contribution from that constituent. Thus $M$ falls on a line between $A$ and $P$, and its position along that line indicates the mixing ratio of $A$ and $P$.

In practice, a soil cover is deployed, and gas samples are taken at the beginning and end of an incubation. $A$ and $M$ are the isotopic compositions of these samples, respectively. $P$ is unknown initially, but must fall somewhere on the ray drawn from $A$ through $M$, and must also fall on the equilibrium curve. Thus, the intersection of the curve and the ray identifies the character of $P$. The atom fraction of the soil N pool, identical (barring fractionation) to that of $P$, is displayed on the horizontal axis. The relative contribution of $P$ to the mix is given by the “travel” of $M$ along the ray (the length of segment AM), divided by the total length of the segment AP. Since the ray has constant slope, the relative contribution can be determined simply from the relative enrichments ($^{15}N$) of $A$, $M$, and $P$ (i.e., the proportion collapses to a single axis).

### Equations

From the concept illustrated in Fig. 2, equations for gas flux and enrichment of source pool can easily be derived by geometric inference. Formally, the isotopic character of a gas mixture and the assumed or measured character of one of its pre-mixing components (e.g., atmospheric N$_2$ already present in a chamber headspace) can be used to calculate both $^{15}a_p$, the atom fraction of the second component (e.g., soil-derived N$_2$), and $d$, the fractional contribution of the second component to the mixture. This is achieved by assuming that the second component is initially in isotopic equilibrium (as stated earlier). The atom fraction of the second component is found by simultaneous solution of an equation for the mixing line

$$^{29}x = C + s^{15}a$$  \[2a\]

and an equation for the equilibrium curve [1b], which results in a quadratic expression:

$$^{15}a_p = [-B ± (B^2 - 4AC)^{1/2}] / 2A$$  \[2b\]

where

$$A = 2$$  \[2c\]
$$B = s - 2$$  \[2d\]
$$C = ^{28}x_m - s(^{15}a_m) = ^{29}x - s(^{15}a)$$  \[2e\]
$$s = (^{28}x_m - ^{29}x)(^{15}a_m - ^{15}a)$$  \[2f\]

and the Subscripts $a$, $p$, and $m$ refer respectively to the initial component (atmospheric N), the second component (soil pool N), and the mixture. $C$ is the intercept for the mixing line, and $s$ is the slope of the mixing line. The relative contribution of N$_2$ from the second component is

$$d = (^{15}a_m - ^{15}a) / (^{15}a_p - ^{15}a)$$  \[3\]

Absolute flux can be calculated from relative flux (relative contribution) by associating some absolute measure with initial or final quantity of headspace gas: for example, if final N$_2$ concentration is 0.8, and chamber volume is 1 L, then a value of 0.01 for $d$ implies evolution of (0.8)(1 L)(0.01) = 0.008 L of N$_2$. Note that a quadratic expression is used to find $^{15}a_p$, which in turn is used to find $d$. Arah (1992) used a quadratic to find $d$ and used $d$ to find $^{15}a_p$. The two systems give identical results. The relevant root in Eq. [2b] can be identified by inspection of Fig. 2 and is necessarily the larger; the other root is identical to $a_s$ in this case (cf., Boast et al., 1988 Eq.
Note again that all mixtures of equilibrium gases necessarily lie under the equilibrium curve. This is true regardless of the number of equilibrium components in a mixture. As an informal proof, consider stepwise additions of many equilibrium components to an accumulating mixture. The first two components create a mixture below the curve; subsequent additions displace the mixture toward some point in the curve, but never past it. The formal proof has been provided by Boast et al. (1988; see Eq. [19]).

**Expectation of Error Due to Multiple Pools**

The utility of the heuristic model outlined above can be illustrated by revisiting the principle assumption of
showed very little scatter about a line with a slope of \(E\). Any method that does not measure disequilibrium methods of N\(_2\) or N\(_2\)O flux analysis (Hauck and Bouldin, 1961; Siegel et al., 1982; Mulvaney, 1984; Arah, 1992). Any method that does not measure disequilibrium methods of N\(_2\) or N\(_2\)O flux analysis necessarily “overshoot” \(P\), intersecting the curve beyond \(P\).

Fig. 2. Visualization of the heuristic model (Eq. [1], [2], and [3]). \(A\) represents the atmosphere and \(M\) represents the sample; positions of both are determined analytically. The ray drawn from \(A\) through \(M\) intersects the equilibrium curve, implicating a pool \(P\) of gas derived from the soil, having an equilibrium distribution of masses.

disequilibrium methods of N\(_2\) or N\(_2\)O flux analysis (Hauck and Bouldin, 1961; Siegel et al., 1982; Mulvaney, 1984; Arah, 1992). Any method that does not measure disequilibrium methods of N\(_2\) or N\(_2\)O flux analysis necessarily “overshoot” \(P\), intersecting the curve beyond \(P\). Thus, \(15a\) will always be overestimated if \(15a > 15a\) (as shown by Boast et al., 1988; and Arah, 1992). It is apparent from Fig. 3 (and from Eq. [3]) that \(d\) is always underestimated: the segment \(A\) calculated is always longer than the segment \(A\) calculated (as per Arah, 1992).

The underestimation can be quantified if the isotopic character of \(P\) is specified. Regarding Arah’s (1992) simulation, it is appropriate to associate \(P\) with the statistical expectations of \(15a\) and \(29x\) for a mixture of equilibrium gases, whose values of \(15a\) are randomly and uniformly distributed on the interval \([g, h]\). With \(P\) thus specified, the expectation of underestimation can be calculated. The answer derived here is independent of the number of contributing gases (pools) because statistical expectation \(E(x)\) is independent of the number of samples. For generality, we take the case where each pool has equal weight. For a random variable uniformly distributed on the interval \([g, h]\),

\[
E(15a) = \frac{(g + h)}{2} \tag{4}
\]

\[
\text{Var}(15a) = \frac{(h - g)^2}{12} \tag{5}
\]

\[
E(15a^2) = E(15a)^2 + \text{Var}(15a) \tag{6}
\]

Considering the dependence of \(29x\) on \(15a\) (Eq. [1b]), the expectation of \(29x\) is given by

\[
E(29x) = E(2 \cdot 15a - 2 \cdot 15a^2) \tag{7a}
\]

\[
= 2E(15a) - 2E(15a^2) \tag{7b}
\]


\[
E(29x) = 2E(15a) - 2\left[E(15a)^2 + (h - g)^2/12\right] \tag{8}
\]

Thus, given a range of enrichments, the expected isotopic character of a random mixture \((15a_{\text{expectation}}, 29x_{\text{expectation}})\) can be determined from [4] and [8]. To calculate the resulting underestimation, an atom fraction \(15a_{\text{calculated}}\) is found by substituting \(29x_{\text{expectation}}\) and \(15a_{\text{expectation}}\) for \(29x\) and \(15a\) in Eq. [2]. A coefficient of estimation \(e\) can be expressed as
Fig. 3. Visualization of underestimation due to multiple soil N pools of differing enrichment. If $P$ is in fact a mixture of equilibrium gases (resulting from multiple pools) then it falls below the equilibrium curve. Thus $P_{\text{calculated}}$ always overestimates $P_{\text{actual}}$, when $^{15}a_p > ^{15}a_a$. Since the segment $A P_{\text{calculated}}$ is longer than $A P_{\text{actual}}$, flux is always underestimated (since flux $= AM / AP$).

$$e = \frac{d_{\text{calculated}}/d_{\text{expectation}}}{(^{15}a_p - ^{15}a_a)/(^{15}a_p_{\text{calculated}} - ^{15}a_a)} \quad [9a]$$

Eq. [9b] can be proven from Eq. [3]. For the case where the range of enrichments is $[0,1]$, $^{15}a_p_{\text{expectation}} = (\gamma)$ by Eq. [4] and $^{28}X_{\text{expectation}} = (1/3)$ by Eq. [8] (Note 2). Interestingly, if the lower bound of the interval $g$ is equal to $Y$ (cf. Arah, 1992), $e = 0.75$, regardless of the range of enrichment; this value depends neither on the value of $\gamma$ nor $h$ (Note 3).

In summary, we have provided a more fundamental interpretation of Arah’s (1992) simulation (Fig. 2a). Our heuristic model for N-gas flux from $^{15}$N-labeled soil shows why underestimation occurs. Our equations reduce the simulation to a relatively straight-forward calculation. We show that even under somewhat less restrictive conditions than Arah’s (i.e., range not specified), the coefficient of underestimation $e$ evaluates to 0.75. This value agrees well with the slope of 0.76 in Arah’s Fig. 2a, which can also be interpreted as an index of underestimation. Simulation is a valuable tool for exploring systems of equations that defy direct solution; the discovery of a direct solution for such a system represents progress. While our solution may not represent any real set of field conditions, it does help predict how field conditions will influence the accuracy of isotopic methods for N-gas flux measurement.

The approach employed above can be used to explore other questions about labeled N$_2$ or N$_2$O fluxes. For instance, Hauck and Bouldin (1961) state that their system gives a value for $^{15}$N that “represents the average isotope content of the material undergoing denitrification over a given period of time.” But the concept of “average” implies that the enrichment is changing with time (e.g., by dilution from concurrent nitrification), and thus the assumption of pool uniformity is violated.

In principle, it makes no difference whether the assumption is applied to space or time. Is change of source pool enrichment with time likely to be a significant source of error in field experiments?

We sampled N$_2$O over a 3.5-d period in April 1998 in a heavily labeled wheat plot (30 kg ha$^{-1}$ as 99% K$^{15}$NO$_3$). Enrichment of the source pool, as inferred from N$_2$O isotope data, dropped gradually from 82 to 72% during this period. Even if this entire drop had occurred during a single incubation, the resulting underestimation would have been negligible. Let $g$, $h$ be [0.7, 0.8] and let $g = 0.003663$. From Eq. [9b] (which invokes others), $e = 0.999$. For comparison, a drop from 80 to 60% during an incubation yields $e = 0.993$. We conclude that error from temporal changes in enrichment for our experiment must have been negligible, and is probably negligible in most cases.

**DISCUSSION**

The heuristic model presented above facilitates the design of N-gas flux experiments and the interpretation of isotopic data for N$_2$ and N$_2$O samples collected over $^{15}$N-labeled soil. It is particularly useful for exploring the problem of underestimation that occurs when N$_2$ or N$_2$O analyzed by mass spectrometry derives in part from a soil pool that is not uniformly labeled: it illustrates how underestimation occurs. We reduced a published simulation of underestimation to a direct calculation based on statistical principles. We showed the general utility of our equations by evaluating a case where enrichment varied over time, rather than in space.

Isotopic methods for measuring N$_2$ and N$_2$O flux have general appeal because they are relatively non-disruptive of soil systems and because they represent the only practical direct method for measuring N$_2$. As isotope ratio mass spectrometry (IRMS) becomes more widely available, use of isotopic methods will continue to grow. Although flux equations and analytical methods have been available for decades, there still exists considerable uncertainty regarding the accuracy of the method when applied in the field. Most of the uncertainty pertains to the necessary assumption that empirical methods result in uniformly labeled soil mineral N pools. Underestima-
tion is expected to result from the failure of the assumption, but the magnitude of the underestimation is difficult to predict.

Our work does not imply the existence of a theoretical method for correcting flux estimates from the field. “Statistical expectation”, as used in our argument, has a precise mathematical definition that is not equivalent to expected error in field measurements, unless field conditions closely match the constraints of the mathematical model. The underestimation calculated by our equations for $e$ will not likely be realized in the field unless (i) the number of pools is large, (ii) the enrichments of the pools are randomly distributed, and (iii) flux is distributed evenly among the pools. We doubt that any of these conditions is likely to be met in field settings, especially the third. Even if the conditions were met, it would seem impossible to know this a priori. Our experience with N2O fluxes shows underestimation to vary within experiments and especially among experiments. When mass spectrometric flux estimates are compared to estimates made by gas chromatography, agreement ranged from 6 to 117% (MS/GC, unpublished data). In a systematic laboratory study of N2O fluxes, Mulvaney (1988) found that differences between MS and GC usually were small (less than 10%) and probably resulted from analytical error. Mulvaney and Vanden Heuvel (1988) found no appreciable difference between MS and GC in the field, unless plots were relabeled. Can pool uniformity ever be assumed? We believe that when added N (labeled) far exceeds preexisting soil N, there is initially only one significant pool, which is practically uniform.

When uniformity of the soil N pool cannot be assumed, it should be demonstrated (e.g., Stevens et al., 1997). However, the conceptual model given here (e.g., Fig. 2 and 3) reminds us that it is not critical to know that the soil pool is uniform; rather it is critical to know the isotopic character of N gas derived from the soil pool. Uniformity merely makes this easy to calculate (by invoking the equilibrium curve as one constraint). The error that attends violation of the assumption of pool uniformity could be avoided if there were alternative means of assessing the isotopic character of the N gas derived from the soil.

It may be possible to assess independently the isotopic character of soil-derived gas. Consider two successive incubations of the same unit of soil. Suppose that, after sampling the headspace at the end of the first incubation, the enclosure is flushed, closed, and spiked with a small amount of $^{15}$N$_2$. The spike has the effect of displacing A$_3$, the base of the ray for the second incubation (Fig. 4). The rays for the two incubations necessarily converge at a point identifying the isotopic character of the total evolved gas, allowing accurate determinations of flux for both intervals (equations are outlined in Note 4). The principal assumption is that the isotopic character of the evolved gas is constant: probably a more robust assumption than pool uniformity. Another assumption is that change in headspace enrichment due to other processes (e.g., equilibration with soil pore space) is negligible. The amount of gas needed for the spike depends on the volume of the headspace and the sensitivity of the mass spectrometer. For N$_2$, such a spike will be relatively expensive until $^{15}$N$_2$ costs drop or sensitivity improves such that smaller changes in $^{15}$N$_2$ abundance become detectable. For N$_2$O, however, for which additions of labeled N such as $^{15}$N$_2$O can readily be measured in a normal atmosphere, this approach is already an option.

Another approach for independently assessing the isotopic character of soil-derived gas is similar to that above. Two consecutive incubations are conducted, but before the second incubation, the headspace is purged of the gas of interest so that the final sample will contain only (mostly) soil-derived gas. This is, again, difficult for N$_2$ because of contamination problems, but is an interesting possibility for N$_2$O.

We believe the heuristic model described here is use-
ful for clarifying principles, for designing experiments, and for evaluating data related to N₂ or N₂O collected over labeled soil. ¹⁵N approaches to measuring N₂ and N₂O fluxes are likely to become more common as associated materials and technology become more readily available. Application of conceptual tools to explore the limits and potential of isotopic methods is warranted.

Note 1

A Cartesian plot of ²⁹x vs. ¹⁵a is graphically identical to a ternary plot of ²⁹x, ²⁵x, and ³⁰x if the ternary plot is bounded by an equilateral triangle, and if the abscissa is expanded by a factor of cos(30)⁻¹ relative to the ordinate (about 15%). Equivalence, but not identity, is preserved even if both conditions are removed.

Consider an equilateral triangle of unit height and horizontal base. Specify three axes that bisect the three vertices and intersect their respective opposite sides at right angles. Scale these from 0 to 1, base to vertex. Beginning with the lower left vertex and proceeding clockwise, assign the axis bisecting the vertex to represent 2₉x, 2₅x, or 3₀x, respectively. Additionally, specify a vertical axis and a horizontal axis (v and h) originating at the lower left vertex and scaled identically to the other three axes. Adopt the term “base” to represent a line normal to an axis, which passes through its origin.

For any given point, what is the relationship between its Cartesian coordinates (h, v) and its ternary coordinates (²⁹x, ²⁵x, ³₀x)? It is clear by inspection that

\[ v = ²⁵x \]

because their bases are collinear. The distance of any point to the base of the h axis can be divided into two portions falling inside and outside the ternary plot, respectively. Trigonometric analysis shows that

\[ h = (³₀x) \cos(30)⁻¹ + (²⁵x) \tan(30). \]

Multiplying both sides by cos(30),

\[ (h) \cos(30) = (³₀x) + (²⁵x) \sin(30). \]

Since all ¹⁵N atoms occur either in the 29x or 30x fractions, and since only half of the atoms in the 29x fraction are ¹⁵N, we can write

\[ 15a = (³₀x) + (²⁵x) (0.5). \]

Noting that sin(30) evaluates to 0.5,

\[ (h) \cos(30) = 15a \quad \text{and} \quad h = (15a) \cos(30)⁻¹. \]

Therefore, a plot of (³₀x) vs. (15a) cos(30)⁻¹ is identical to a ternary plot of ²⁹x, ²⁵x, and ³₀x. The factor cos(30)⁻¹ merely scales the abscissa, and is largely irrelevant. The plot ²⁹x vs. 15a also emulates a valid ternary plot, albeit with a compressed base.

Note 2

To illustrate this, we calculated the isotopic character of 100 simulated gas mixtures by mass balance. Number of pools was randomized on the interval [2, 50]. Enrichment as well as relative weights of pools were randomized on the interval [0,1]. When plotted, the isotopic characters of the mixtures clustered around the coordinates (1/2, 1/3) for (³₀a, ²⁵x). Mean coordinates for all values were (0.497, 0.334). Mean coordinates for 100 000 simulated mixtures were (0.5004, 0.3336).
\[ k_1 = (4h^2 - 8y^2 + 4h\gamma + 4h^2 + 4\gamma - 8h\gamma)[12(h - \gamma)] = (2h^2 - \gamma - h\gamma)/[3(h - \gamma)] = (\gamma + 2h)/3 \]

\[ k_2 = (4h^2 - 8y^2 + 4h\gamma - 4h^2 - 4\gamma + 8h\gamma)/[12(h - \gamma)] = \gamma \]

For \( k_2 \):
\[ e = (i - \gamma)/(k - \gamma) \text{ (undefined)} \]

For \( k_1 \):
\[ e = (i - \gamma)/(k - \gamma) = [(h + \gamma - 2\gamma)/2]/[(\gamma + 2h)/3] = [(h - \gamma)/2] \times [3/(2h - 2\gamma)] = [3(h - \gamma)]/4(4h - \gamma) = 3/4 \]

**Note 4**

Using the symbology in Fig. 4, fractional contribution of the soil pool to the final mix is calculated as

\[ d_x = (5^a_{Mn} - 5^a_{Aa})(5^a_p - 5^a_{Aa}) \]

where \( d \) is the fractional contribution, \( n \) is the incubation number, \( 1^a \) is the enrichment of the sample, \( A \) is the initial chamber headspace, \( M \) is the final mix, and \( P \) is the soil-derived component. The enrichment of \( P \) can be found by solving for the intersection of the two rays as follows:

\[ (s_1)(1^a) + C_1 = (s_2)(1^a) + C_2 \]

\[ (1^a)(s_1 - s_2) = C_2 - C_1 \]

\[ 1^a = (C_2 - C_1)/(s_1 - s_2) \]

where \( s \) is slope, \( C \) is intercept (by analogy to Eq. [2a]) and the subscripts reference the two incubations.

Slope is calculated as

\[ s_n = (2^a_{Mn} - 2^a_{Aa})(2^a_{Mn} - 2^a_{Aa}) \]

where \( 2^a \) is the mole fraction of singly-substituted molecules.

Intercept is calculated as

\[ C = 2^a_{Mn} - s(2^a_{Mn}) - 2^a_{Aa} - s(2^a_{Aa}) \]

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