

# **Modeling the dynamics of stable-isotope ratios for ecosystem biogeochemistry**

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## Introduction

Models incorporating stable isotopes have been used for a number of purposes in ecosystem science and biogeochemistry. The primary motivation for their development and application has been to interpret the results of field and laboratory studies. In order to interpret the temporal or spatial patterns observed in isotopic ratios in field samples, investigators require mathematical representations of fluxes among or mixing among conceptual pools. Additional uses of these models include the planning of isotope experiments and the generation of hypotheses (Schimel 1993, Currie and Nadelhoffer 1999). A less common but promising application is the use of isotopically-tested models to simulate other quantities of interest, such as energetic fluxes, interactions among element cycles, and ecosystem responses to global change (Tietema and Wessel 1992, van Dam and van Breemen 1995, Currie 2003, Currie et al. 2004).

Isotopically-explicit models cover a broad range of complexity and a broad spectrum of mathematical approaches. At the simplest end of the spectrum lie mixing models and isotope dilution or “pool dilution” models having one or two conceptual pools (Davidson et al. 1991). These models can be expressed as algebraic equations and typically solved analytically. The fundamental principles behind the pool-dilution equations were articulated over 50 years ago (Kirkham and Bartholomew 1954). More complex models developed in the past two decades rely on many of the same assumptions of simple pool dilution models, such as that each conceptual pool is well-mixed and isotopically homogeneous and that inputs to a pool can alter its isotopic ratio but outputs from a pool can not. It has been conceptually straightforward to incorporate these principles into complex process models with numerous pools and fluxes, nonlinear interactions, tracer recycling, and non-steady-state dynamics. However, developing the mathematics and computer programs to do so has been an intellectual and computational challenge. One aspect of the challenge is that enhanced physical detail, not originally needed in the conceptual model to describe bulk elemental flows, may need to be added to a model to provide any possibility of simulating isotopic mixing. When added to the algorithms needed to simulate bulk elemental flows, the computer coding algorithms required to carry out isotopic mixing computations can be substantially more elaborate. They also require more sophisticated testing and need to work properly to a higher number of significant digits (e.g. 6 to 8) with small numbers close to zero. Another challenge arises in the choice of a model time step. A relatively coarse time step (one month, for example) may be suitable for modeling bulk elemental movements and transformations. A much finer time step may be needed to simulate isotopic mixing in pools that turn over rapidly or that attain values close to zero repeatedly on finer time scales.

Analytical solutions, unfortunately, are obtainable in only a minority of the interesting and important problems in the study of nutrient and carbon cycling (e.g. Riha et al. 1986). This limitation is inherent in applied mathematics more broadly. An illustrative example is the so-called “three-body problem” in physics. In this well-known problem, gravitational interactions can be written as a set of equations for any number of interacting celestial bodies (planets, moons, satellites, or stars), but analytical solutions can be found only in cases where no more than two bodies interact – one planet and the Sun, for example. When a third body is introduced (a moon, satellite, or another planet), the equations of motion cannot be solved analytically. Numerical methods must be used and computers have made such problems tractable. In an analogous way, when isotopes are allowed to recycle among ecosystem pools and when there are more than two pools in the conceptual model, we essentially have the three-body problem and we require numerical or algorithmic approaches.

Intermediate in the spectrum of complexity among stable-isotope models are those expressed as sets of differential equations. Under highly restricted assumptions (such as steady-state and no tracer recycling), the simplest of these can be solved analytically. In other cases, numerical integration techniques are used. At the most complicated end of the spectrum lie dynamic biogeochemical models expressed as computer algorithms. Driven by the increasingly widespread laboratory analysis of stable-isotope ratios in field studies, several complex process models have now been developed that include stable-isotopic ratios in ecosystem pools as state variables.

This chapter reviews the present state and recent advances in the mathematical modeling of stable isotopes for research in ecosystem biogeochemistry. I draw primarily on models that explicitly include the isotopes of nitrogen (N) and carbon (C). However, many of the principles and techniques described here can potentially be extended to the modeling of stable isotopes generally. By ‘model’ I include all manner of mathematical formulations from the simplest algebraic equations to elaborate computer models. Virtually all interpretations of stable-isotope data require equations that are derived from a conceptual framework of pools or end-members together with fluxes or mixing. I begin with a review of principles important in designing model-data comparisons, then describe modeling frameworks across the spectrum of mathematical approaches, and close with a discussion of qualitative behavior and uncertainty in complex models.

## Conceptual and mathematical models

The conceptual model of an environmental system or subsystem stands between the natural system of interest and the mathematical model used to interpret observations (Fig. 1). In planning an experiment or in interpreting data, an investigator first establishes a conceptual model that simplifies nature by introducing some assumptions and approximations. In interpreting stable-isotope data, investigators translate their conceptual model into sets of equations that can be analyzed analytically or into computer algorithms that can be run and studied numerically.

Inconsistencies are likely to exist between both (a) the natural system and the conceptual model and (b) the conceptual and mathematical models. A key part of the modeling activity is to understand and to minimize such inconsistencies and to understand the tradeoffs to be made. While important consistencies can be achieved, some amount of inconsistency seems unavoidable. Investigators judiciously choose which aspects of a natural system to represent best in a conceptual model while allowing known fictions to exist in other aspects. Hobbie et al. (1999) have pointed out that many conceptual fluxes in biogeochemistry are, in reality, combinations of several chemical and physical processes. For example the uptake of  $\text{NH}_4$  by plants involves the animation of glutamate to form glutamine, which is then used to animate other compounds in plants. The question to ask in assessing a conceptual model is not whether it is completely realistic (meaning it faithfully represents the natural system in every detail). Rather, one question to ask is whether a model is reasonably faithful to the present understanding of biogeochemical processes while being *useful* in a particular context for advancing the goals of the research. To be useful, a model should have enough realism and complexity to capture the key interactions to be addressed in a research project, but should eschew gratuitous detail.

In choosing tradeoffs to make in model construction, one should consider how the model will be used in conjunction with field or laboratory experiments. Models can make strong contributions to empirical research when they are tailored to the design of field experiments. For example, imagine an experiment in which a  $^{15}\text{N}$  tracer was to be added to a soil surface, then at a later time mineral soil samples were taken and  $^{15}\text{N}/^{14}\text{N}$  ratios measured in sorted fractions. The conceptual model of the pools and fluxes of N should guide the construction of both the field experiment and the model (Fig. 1). Close consistency in the definitions of compartments, or pools, is key (Schimel 1993). If mineral soil samples from the field study were to have living fine roots separated out for analysis but dead fine roots composited with mineral soil organic matter (SOM) for analysis, the model should be designed to allow a direct comparison between model results and field data. If, in this example, the model pooled living and dead fine roots together, then a direct comparison of  $^{15}\text{N}/^{14}\text{N}$  ratios between the modeled and the field-sampled pools would be impossible. In this example the mismatch is obvious; in other cases they are more subtle. To take this example further, investigators studying the forest soil would need to consider whether microbial biomass will be implicitly included in soil pools or explicitly separated; whether detrital layers in the forest floor will be sorted in the field study according to physical layering or to presumed degree of decomposition, and so on. Careful consideration should be given to designing a conceptual model that facilitates both (a) addressing the objectives of the field study and (b) guides the construction of a mathematical model that will be useful and testable using the field data to be obtained.

Some assumptions are common to all biogeochemical models because they are part of the systems paradigm that is fundamental to the field. One such approximation is that each pool in a conceptual model represents a homogeneous, well-mixed pool with a single overall rate of export or turnover. In most cases this approximation is probably false. Biogeochemical fluxes and transformations are dynamic at multiple scales and “hot spots” of activity exist potentially even at fine scales. Within organisms and tissues, isotopes may not penetrate evenly and an organism or a tissue may not behave as a single well-mixed pool (Conover and Francis 1973, Schimel 1993). Similarly, the material we define as mineral soil organic matter, whether we conceive of it as one pool with a single turnover rate (Aber and Federer 1992), two pools with distinct turnover rates (Clark 1977, Currie et al. 2004), or three pools with distinct turnover rates (Parton et al. 1987), may in reality lie closer to a continuum of materials with a continuum of turnover rates (Agren and Bosatta 1996). Future modeling efforts could address means to incorporate a continuum of pool turnover rates into an isotope mixing model, but to date, models have incorporated discrete pools with discrete rates of turnover.

## Designing consistent model-data linkages and comparisons

### An additional dimension of observation

A ratio of stable isotopes such as  $^{15}\text{N}/^{14}\text{N}$ , which can be measured with high precision using mass spectroscopy, offers intrinsic benefits as a model state variable. Isotopic ratios are sensitive to gross fluxes of the element into or out of an ecosystem pool, even where net fluxes may be small, may be zero, or may flow in the opposite direction to a gross flux of interest (Schimel 1993). A simple model example using steady-state N fluxes and pool sizes in two conceptual pools (shown in Figs. 2 and 3) illustrates this principle. In the model results shown in both of these figures, the net flux of N from pool  $P_b$  to  $P_a$  is  $0.2 \text{ g N m}^{-2} \text{ timestep}^{-1}$ , and a  $^{15}\text{N}$  tracer with  $\delta^{15}\text{N} = 1000\text{‰}$  is introduced to pool  $P_a$  at time zero. Note that a field measurement of net N fluxes, lacking isotopic information, would be unable to distinguish between the two cases depicted (Figs. 2 and 3). The  $^{15}\text{N}$  tracer, in contrast, when introduced to pool  $P_a$  allows us to distinguish between these two cases because in the model results we see the  $^{15}\text{N}$  appear in pool  $P_b$  quite rapidly, even though the *net* flux of N is from pool  $P_b$  to  $P_a$ , or opposite the direction of flow of the  $^{15}\text{N}$ .

Investigators do not analyze or model an isotopic ratio in isolation. Quite the contrary: the ratio  $^{15}\text{N}/^{14}\text{N}$ , for example, is viewed as an additional state variable that is measured and modeled together with an existing array of state variables that includes measures of molecular forms of N or total N. It is of limited value to compare isotope ratios between model and data if modeled pool sizes or fluxes of N, C or organic matter are widely unrealistic, or if the degree of realism in these is inadequately known. Furthermore, such comparisons would miss the value of using isotopic ratios because they would replace one state variable (elemental pool size) with another (isotopic ratio in a pool), rather than combining state variables to gain an increased dimension of comparison.

Many pools of an element in which stable-isotope ratios are measured are operationally defined. Investigators often analyze isotopic ratios and elemental concentrations in subsamples that have undergone other extractions or separations. For example, the ratio  $^{15}\text{N}/^{14}\text{N}$  might be measured in extractable  $\text{NH}_4$  from soils (together with  $\text{NH}_4\text{-N}$  concentrations), in extractable  $\text{NO}_3$  from soils (together with  $\text{NO}_3\text{-N}$  concentrations), and in the inextractable soil residue (together with total N concentrations in the residue). As another example, the  $^{15}\text{N}/^{14}\text{N}$  ratio (together with N concentrations) might be measured in subsamples of fine-root litter, foliar litter, and unidentifiable detritus each sorted by hand from a soil organic horizon.

When a  $^{15}\text{N}$  tracer is added and followed over time, this can be viewed as labeling a temporal cohort of N inputs to the system. Investigators can then follow that cohort of N inputs as it is partitioned initially and then as it redistributes within the ecosystem over time. For example, Nadelhoffer et al. (2004) applied  $^{15}\text{NH}_4$  and  $^{15}\text{NO}_3$  tracers to forest floors at the Harvard Forest, Massachusetts over a 16-month period in 1990-1991 and sampled numerous vegetation and soil pools over the subsequent 8-year

period. Over this time period, differences in values of  $\delta^{15}\text{N}$  from natural-abundance values in vegetation and soil pools reflected the location of the “1990-1991-Nitrogen” as it moved through the ecosystem. To enhance this interpretation of the labeling experiment, the label should be added in a molecular form and in a manner that mimics natural (or ambient) N inputs to the system or to a particular pool. It is also important to consider that a labelled cohort of N will move through the ecosystem in a way that reflects the state of the system at the time of the labeling. For example, suppose a hypothetical field manipulation to increase the downward transport of N through the soil, perhaps through irrigation, began in the year 2000. The initial partitioning of a label applied to the forest floor in 1999 is likely to be very different from that of a similar label applied in 2001 once the irrigation treatment were under way. The redistributions of these hypothetical labels over 10-year periods would reflect the differences in this initial partitioning.

### **Model-data comparisons**

Because the logical basis of process-based or causative models is deductive, model realism must be checked against empirical observations. This may involve formal or informal approaches to model testing, verification, or validation. These terms are used with wide ranges in meaning (Oreskes 1994, Gardner and Urban 2003); I simply point out that any such efforts require direct model-data comparisons. Additionally, as noted above, the primary motivation in developing stable-isotope models has been to aid in the interpretation of field and laboratory data, which necessarily requires an assumed correspondence between measured and modeled quantities.

Although we ultimately seek to understand systems in nature, it is through observational methods and techniques (with their inherent limitations) that we use empirical data to assess models or use models to interpret observational data (Oreskes 1994). In this sense, *observables* stand between nature and our mathematical models (Fig. 1). Whether for model testing, model parameterization, model calibration, or model use to interpret observations, it is important to keep this in mind when designing or finding key points of model-data comparison. Observables do not necessarily reflect our current conceptualizations of fundamental processes in the ecosystems we study; often times they instead reflect operational definitions. Comparisons between observed and modeled quantities may be mismatched or ill-defined. This consideration enters any model-data comparison (Magid et al. 1997), but it is amplified in the case of isotope models. Both an elemental pool size and its isotopic signature enter into a calculation of tracer recoveries (explained below) and both may be mismatched between a modeled quantity and the corresponding field measurement. Given these considerations, model-data comparisons should be constructed not necessarily to represent fundamental processes. Instead, a close correspondence is needed between a quantity as modeled and the same quantity *as measured by observational techniques*.

Ecosystem biogeochemistry is rich in operationally-defined quantities, but these may or may not match the pools and fluxes in our conceptual models. For example, during decomposition of plant litter, C is partly respired to  $\text{CO}_2$  and partly transformed to a quasi-stable material, humus. Humification is often conceived, quantitatively, as occurring to 15-20% of the initial litter mass for foliar and fine-root litter (Aber et al. 1990). This conceptual model works well in computer models: when a certain portion of initial mass remains, the material is passed to a more stable pool with a lower turnover rate. But a problem arises when we try to compare the modeled size of the humus pool, or its isotopic signature, with field observations. Field definitions of components of the soil organic horizon are based on rubbed fiber content (an operational definition), or on whether the initial source of the material is identifiable as to leaves, needles, roots, bark, and so on, or not so identifiable (an operational definition), or by horizontal layering of layers in the horizon (also an operational definition). None of these definitions used in the field matches the conceptualization upon which the model operates in this example, i.e. the definition of humus as what remains after 80 to 85% of the initial litter mass has been lost. Rather than being a clear point of model-data comparison, the size of the humus pool or its isotopic signature is in this case a vague comparison and involves the entry of yet another conceptual model (the correspondence of ‘percent of initial mass remaining,’ in the model, to ‘identifiable subhorizon,’ in the field). This corollary conceptual

model is not explicit and not falsifiable in this context. Mismatches like this occur to varying degrees in many, if not most, points of model-data comparison in isotope models. Examples abound, including operational definitions of plant-available nutrient pools (e.g. through buried-bag field incubations, Hart et al. 1994), operational definitions of leaching fluxes (material passing through a filter of a certain pore size, Lajtha et al. 1999), and the list goes on. Together these seriously hamper our ability to view model-data comparisons as firm quantitative tests or interpretations.

In some cases models can have internal pools, parameters, or fluxes designed to match precisely to operationally-defined quantities from a particular field study. This, however, can result in a loss of generality or a substantial increase in modeling effort to include multiple operationally-defined options for the model user. Currie et al. (2004) addressed this problem in the TRACE model (Tracer Redistributions Among Compartments in Ecosystems, Currie et al. 1999) by allowing isotopic tracers in dead fine-root tissue to be accounted for under to three different definitions used in field studies: either together with live roots, or composited together with soil organic matter, or as its own pool separate from live roots and from soil organic matter. To interpret isotopic tracer recoveries in a field study, the user of the TRACE model chooses the set of pool formulations that best matches the field-study design. This solution, however, has drawbacks. It requires a large amount of computer code dedicated to carrying out 'bookkeeping' calculations in three different ways, each needing to be thoroughly tested and thoroughly integrated with the rest of the model.

### **Metrics: stable isotope ratios, mass balances, tracer percent recoveries**

Several metrics are available to compare modeled ratios of isotopes against observations. In modeling natural-abundance isotopic ratios, investigators typically use the familiar  $\delta$  notation. This is a widely recognized quantity (Lajtha and Michener 1994) that is based on the mass ratio of stable isotopes in a sample, or, when averaged across replicate samples, in an organism tissue or ecosystem pool at a point in time. It is the metric typically reported in mass-spectroscopic results and can be easily calculated in a computer model. Note that  $\delta$  is an intensive variable; the size of the elemental pool does not enter into its calculation. Other intensive variables that are related solely to the isotopic ratio include the ratio itself (e.g.  $^{13}\text{C}/^{12}\text{C}$ ) or the atom % of the less common isotope (e.g. atom %  $^{13}\text{C}$ ).

In tracer studies, investigators are interested in the relative apportionment of the tracer into various compartments in an organism, subsystem, or ecosystem. This information is not conveyed by the  $\delta$  notation or atom % notation alone. A small pool receiving a small amount of enriched  $^{15}\text{N}$  tracer could show a large change in  $\delta^{15}\text{N}$  or atom %  $^{15}\text{N}$ , whereas a large pool receiving a large amount of the tracer could show a small change in  $\delta^{15}\text{N}$  or atom %  $^{15}\text{N}$ . To interpret tracer studies, in any ecosystem pool in which the isotopic ratio,  $\delta$  value (e.g.  $\delta^{15}\text{N}$ ), or atom % (e.g. atom %  $^{15}\text{N}$ ) is reported, the pool size of elemental mass (e.g. total N mass in  $\text{g N}/\text{m}^2$ ) must also be scaled up and reported (e.g. Nadelhoffer et al. 2004).

In a mass-balance approach to interpret recoveries of enriched tracers, investigators combine an extensive variable describing an elemental pool size with an intensive variable related to its average isotopic character. Extensive variables (e.g. mass or volume) are those that scale with the size of a pool, whereas intensive variables (e.g. pH or temperature) are independent of pool size. For example, one could combine bulk N mass in a pool (an extensive variable) with either its  $^{15}\text{N}/^{14}\text{N}$  ratio or atom %  $^{15}\text{N}$  (each intensive variables) to calculate  $^{15}\text{N}$  mass in each pool before and after the introduction of a  $^{15}\text{N}$  tracer (Zak et al. 2004.) Such mass-balance calculations do scale directly with the elemental pool size and thus do convey the relative apportionment of enriched tracers among compartments in a soil, plant, or ecosystem. Many investigators divide a mass-balance expression of tracer recovery in each pool by the mass of isotopic tracer (above background) applied in the study to express recoveries as "percent recovery" of the tracer. Several different formulations of percent recovery have been used (Nadelhoffer and Fry 1994, Hart et al. 1994, Hauck et al. 1994). Currie et al. (1999) defined  $PR^{15}\text{N}$ , the percent of the

$^{15}\text{N}$  tracer mass (above background) that is recovered (above background) in a particular ecosystem pool, or compartment ( $C_i$ ), at a particular point in time ( $t$ ) as follows:

$$PR^{15}\text{N}(C_i, t) = \frac{N_{C_i}(t)(\text{atom}\%^{15}\text{N}_{C_i}(t) - \text{atom}\%^{15}\text{N}_b)}{A(t-t_0)(\text{atom}\%^{15}\text{N}_a - \text{atom}\%^{15}\text{N}_b)} \quad (1)$$

where  $N_{C_i}(t)$  is the amount of N [ $\text{g}/\text{m}^2$ ] in  $C_i$  at time  $t$ ,  $A(t-t_0)$  is the sum of N amendments ( $\text{g}/\text{m}^2$ ) to time  $t$ ,  $C_i$  is an ecosystem compartment, and the ‘a’ subscript denotes amendment, ‘b’ denotes background.

This quantity, the percent recovery of enriched tracers, has proven useful both in expressing field-study results alone and for direct comparison against model results. Because it includes the amount of tracer added to time ( $t$ ) and recovered at time ( $t$ ), it can be used in the midst of a study in which tracers are added over several applications or added continually. It has been useful in following temporal redistributions of isotopic tracers over long time periods (Currie et al. 2004). Because it corrects for pre-existing differences in natural abundance and corrects for different strengths or amounts of tracer added, it is useful in making succinct and intuitive comparisons among different field treatments (Currie and Nadelhoffer 1999). For example, in a large-scale field study at the Harvard Forest, Massachusetts (USA), Nadelhoffer et al. (1999, 2004) used  $^{15}\text{N}$  inputs to label both ambient (untreated) and fertilized (N-amended) plots with tracers differing in values of atom %  $^{15}\text{N}$ . Comparisons of  $\delta^{15}\text{N}$  values reveal instrument sensitivity and standard errors of groups of samples. Comparisons of percent recovery of the tracers allowed differing patterns of tracer movement among forest types and treatments to be clearly compared and conveyed.

Other benefits of  $PR^{15}\text{N}$  for model-data comparisons arise from its inclusion of extensive pool sizes and its ability to be summed directly. If an observational dataset and a model used slightly different sizes for a pool of N – for example, the stock of N in fine roots expressed in  $\text{g N}/\text{m}^2$  – this mismatch could be missed in a model-data comparison of  $\delta^{15}\text{N}$  values. But a comparison of  $PR^{15}\text{N}$  values would be sensitive to model-data differences in both  $^{14}\text{N}/^{15}\text{N}$  ratios and sizes of the pools being compared. Additionally, quantities of  $PR^{15}\text{N}$  can be summed. For example, values of  $PR^{15}\text{N}$  in current-year needles and in prior-year needles can be summed to express  $PR^{15}\text{N}$  in all needles; similarly,  $PR^{15}\text{N}$  values in separate soil horizons can be summed to express  $PR^{15}\text{N}$  in the solum. Percent recovery of tracers is thus a convenient quantity for constructing budgets in a tracer study.

The expression for  $PR^{15}\text{N}$  (eq. 1) includes subtraction of natural-abundance values of atom %  $^{15}\text{N}$  in each pool. This introduces some uncertainty because one needs to measure background values of atom %  $^{15}\text{N}$  prior to the label application, or in a separate unlabelled plot and assume that these represent background values of atom %  $^{15}\text{N}$  in the labeled plot over the period of sampling.

### **Model results sum and balance, but field results may not**

A source of disparity in model-data comparisons is often evident when tracer recoveries are summed over the components of a system. Model results are constrained by a mathematical mass balance of the isotope. If a mathematical model is logically self-consistent, its values of percent recovery of tracers (e.g.  $PR^{15}\text{N}$ ) will sum to 100% after accounting for system losses. Field observations, in contrast, are typically scaled up from fine-scale samples and may not quantitatively account for all of the isotope in the system under study. Nutrient and carbon budgets based on field observations need not exhibit mass balance (Lajtha 2000) and field-measured values of percent recoveries of tracers typically do not sum to 100%. In the field, different components of a nutrient budget may be measured by different teams of investigators at different locations or at different scales. In some cases, experimental uncertainty is large; in other cases, some pools may be incompletely sampled or omitted from the field study altogether. For example, mineral soils may be sampled to a limited depth (10 to 20 cm), limiting the model-data comparison of percent recovery of a tracer in the solum.

Different pool sizes and fluxes of N in a field study may be measured at different points in time, introducing additional uncertainties into mass-balance calculations based on field data. For example, foliar N content and  $^{15}\text{N}/^{14}\text{N}$  ratio might have been measured in July of 2000, while the forest floor N content and  $^{15}\text{N}/^{14}\text{N}$  ratio might have been measured in October of 2001. Under such circumstances, model-data comparisons are a strength in helping to interpret field data. Each of the quantities the model simulates are quantified in each time step; a process model can simulate values for N content and  $^{15}\text{N}/^{14}\text{N}$  ratios in foliage in July of 2000 and the soil O horizon in October of 2001 that are consistent with each other, consistent with the conceptual model of system dynamics, and able to be compared directly against field observations that are separated in time.

Another consideration in making model-data comparisons is that in some sense one is comparing one model against another. Scaling up observations from individual samples to ecosystem pools requires a conceptual and mathematical model; this may be expressed as sets of equations in a spreadsheet. Additionally, it is important to realize that some ostensibly ‘measured’ numbers are in better considered as modeled quantities. Nutrient uptake by vegetation provides an example. Field investigations do not measure fluxes of nutrient uptake; instead, tissue concentrations of nutrients are measured, and a conceptual model and set of equations is employed to calculate fluxes of nutrient uptake. In such cases, consistency in model-data comparisons will be aided if investigators use the same set of scaling assumptions in the process model and the spreadsheet equations used to scale up observations. Close collaboration between modelers and field investigators at each stage of the research is advisable because it furthers such consistency.

## Principles and techniques of stable isotope modeling

### Algebraic mixing models

I outline two types of models commonly formulated as algebraic equations: mixing models and isotope dilution or “pool dilution” models. In the simplest form of mixing model, the isotopic ratio in a measured pool  $R_M$  is considered to result from a mixture of two ‘end member’ ratios  $R_A$  and  $R_B$  (Fig. 4). ( $R$  refers to an isotopic ratio such as  $^{13}\text{C}/^{12}\text{C}$ .) In equation (2),  $a$  and  $b$  are parameters representing the weighted contributions of end-member ratios  $R_A$  and  $R_B$ , respectively:

$$R_M = aR_A + bR_B \quad (2)$$

If one stipulates that the parameters  $a$  and  $b$  are proportions such that  $0 \leq a \leq 1$ ,  $0 \leq b \leq 1$ , and  $a + b = 1$ , then there is a unique  $(a, b)$  pair for a given  $R_A$ ,  $R_B$ , and  $R_M$ . Simple substitution of  $(b = 1 - a)$  into eq. (2) and solving for  $a$  yields the following:

$$a = \frac{R_B - R_M}{R_B - R_A} \quad (3)$$

This two-component mixing model has been widely used in several types of applications. These have included isotopic analysis of food webs, analysis of strontium isotopes to determine relative inputs from weathering versus atmospheric deposition (Vitousek et al. 2003), analysis of the contributions of C3 versus C4 plant sources to soil organic carbon (Balesdent et al. 1987, 1988), and analysis of the contribution of biologically-fixed N versus soil N to plants. For the latter case, Shearer and Kohl (1986) developed the following equation (Amundson and Baisden 2000):

$$a = \left( \frac{\delta^{15}\text{N}_S - \delta^{15}\text{N}_P}{\delta^{15}\text{N}_S - \delta^{15}\text{N}_F} \right) \quad (4)$$

where  $a$  is the proportion of plant N deriving from N fixation and  $\delta^{15}\text{N}_S$ ,  $\delta^{15}\text{N}_P$ , and  $\delta^{15}\text{N}_F$  refer to the  $\delta^{15}\text{N}$  values of soil N, plant N, and fixed N, respectively. Equation (4) is analogous to eq. (3) when the isotopic signature of plant N is viewed as the mixture of the isotopic signatures of the two end-members soil N and biotically-fixed N. Schimel (1993) provided an alternative equation for this mixing model and indicated that it may be preferable to use isotopic ratios (as in eq. 3) rather than  $\delta$  values. An analogous equation to calculate the relative inputs of C3 versus C4 vegetation to soil organic matter, based on a 2-component isotopic mixing model for natural-abundance differences in C isotopes in C3 versus C4 plants, was developed by Balesdent et al. (1987, 1988) and reproduced in Schimel (1993), Wolf et al. (1994), and Amundson and Baisden (2000).

When isotopic signatures of two elements are available simultaneously (for example  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , or  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ ), then a three-component mixing model can be used; a unique solution can be found for the contributions of each of three end-members to the two measured isotopic ratios in the mixed pool or sample. In this case, where  $R(N)$  denotes the isotopic ratio  $^{15}\text{N}/^{14}\text{N}$ ,  $R(C)$  denotes the ratio  $^{13}\text{C}/^{12}\text{C}$ , subscripts  $A, B, C$  refer to the three end-members and subscript  $M$  to the measured sample, and  $a, b, c$  refer to the mixing proportions, the three equations and three unknowns  $a, b, c$  are written as in equation (2), which can be reduced algebraically for  $a, b$ , and  $c$  through substitution:

$$\begin{aligned} R(N)_M &= aR(N)_A + bR(N)_B + cR(N)_C \\ R(C)_M &= aR(C)_A + bR(C)_B + cR(C)_C \\ a + b + c &= 1 \end{aligned} \tag{5}$$

Uncertainty enters into these two or three-component mixing models from several sources. One source is the variabilities, which could be expressed as standard errors of measurement, of the isotopic ratios within each of the end members and within the mixed pool or sample. Uncertainty also enters in the structure of the model, meaning a two-component model may approximate a situation in which three or more isotopically distinct sources exist in nature. Another source of uncertainty arises from the possibility of isotopic fractionation occurring as part of the mixing process or subsequent to the mixing process. Examples of the former include during diffusion or advection through a soil or during uptake by vegetation. An example of fractionation subsequent to the mixing process occurs when the mixed quantity is the isotopic signature in an organism. There are typically differences in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between organisms and their food sources, due to fractionation during metabolism and excretion. Among types of organisms in a trophic level, the variabilities in the net effects of such fractionation can be on the same order of magnitude as the effect itself, limiting the precision with which a mixing model can be used for food sources (Ponsard & Arditì 2000).

### **Algebraic models of pool dilution**

Isotope dilution or “pool dilution” models are so named because any flux of non-enriched element into an artificially-enriched pool or compartment will dilute the enrichment of the pool. Relatively simple pool-dilution models can be drawn with 1-3 elemental pools or compartments together with associated fluxes. These models can be expressed as simple algebraic or differential equations. Typically, unique algebraic solutions can be found only when several assumptions are made. These include the assumption that total elemental fluxes among pools are in steady-state, or that the enriched tracer does not recycle among pools over the time of the study. Solutions describe values of elemental fluxes that are consistent with the assumptions together with the observed changes in isotopic enrichment of the pools at points in time following the addition of an enriched tracer (thus the mathematical models are used to interpret the observations in terms of conceptual pools and fluxes). This analytical modeling approach is widely used in N-cycling research to calculate gross N transformations in soil such as gross  $\text{NH}_4$  mineralization, gross nitrification, or gross  $\text{NH}_4$  and  $\text{NO}_3$  uptake by soil microorganisms. Methods for pool dilution and mass-

balance techniques for fine-scale soil cores and for plot-level studies are standard procedures that are widely covered in the literature (Hart et al. 1994, Robertson et al. 1999, Schimel 1993).

Tietema and Wessel (1992) assessed uncertainties and errors introduced by the assumption that enriched tracers do not recycle. They tested two simultaneous single-pool models of pool dilution of  $\text{NH}_4$  and  $\text{NO}_3$  by comparing the algebraic solutions to the results of a computer simulation. Using standard techniques,  $^{15}\text{NH}_4$  and  $^{15}\text{NO}_3$  were added separately to forest soil cores in the laboratory from three forest sites on each on four dates. Standard equations of production and consumption of  $\text{NH}_4$  and  $\text{NO}_3$  (Kirkham and Bartholomew 1954) were first used to calculate gross rates of  $\text{NH}_4$  mineralization,  $\text{NH}_4$  immobilization, nitrification, and  $\text{NO}_3$  immobilization. According to these authors, the equations contained the following five assumptions: (a) that fractionation was negligible, (b) that  $\text{NH}_4$  and  $\text{NO}_3$  pools after enrichment with  $^{15}\text{N}$  were each homogeneous, (c) that the natural-abundance ratios of  $^{15}\text{N}/^{14}\text{N}$  did not vary among pools at the start of the experiment, (d) that gross N transformation rates were constant for the duration of the experiment (i.e. that nutrient fluxes were in steady-state) and (e) that there was no recycling of enriched  $^{15}\text{N}$  tracers (Tietema and Wessel 1992). (Note that the absence of tracer recycling is a necessary approximation made by all algebraic models as well as most differential-equation models). These authors then constructed a numerical (computer) simulation model that included each of the first four assumptions but allowed  $^{15}\text{N}$  tracer recycling. The simulation model calculated higher gross rates of N transformation, on average, than the algebraic model. In most cases the simulation model estimated rates higher than the algebraic equations by 20% or less; in a few cases (particularly where gross fluxes were small), differences between the models were as high as 100%. When this source of uncertainty was combined with statistical measures of confidence in observations of  $^{15}\text{N}/^{14}\text{N}$  (such as standard errors), overall uncertainties were even greater. The authors concluded that, because of its limiting assumptions, the standard algebraic model could reveal semi-quantitative insights into differences in gross N transformation among soils and dates, as opposed to quantitative rate calculations.

### Differential equation models

Formulations relying on differential equations have the benefit of making use of widely understood conceptual archetypes but, at the same time, introduce conceptual limitations. Such models are often designed for use in a specific study, for example to infer rates of fractionation from a particular or generalized pattern in natural-abundance observations. Schimel (1993) described a differential-equation approach used by Alperin et al. (1988) to calculate a discrimination coefficient for C during methane oxidation based on vertical patterns of carbon isotopes and hydrogen-deuterium in marine sediments. Key assumptions were that diffusion dominated methane movement and that observed vertical gradients in methane were in steady state. Somewhat similarly, Amundson and Baisden (2000) developed a differential-equation model to explain vertical patterns of C isotopes in soil organic matter. This example is informative because it illustrates both the power and limitation of the differential analytical approach.

Amundson and Baisden (2000) began with a partial differential equation describing changes in the concentration of each isotope of soil organic C over time (t) and soil depth (z):

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial z^2} - v \frac{\partial C}{\partial z} - kC + f_d \quad (6)$$

$$\frac{\partial C^*}{\partial t} = D \frac{\partial^2 C^*}{\partial z^2} - v \frac{\partial C^*}{\partial z} - \alpha kC^* + f_d R_1 \quad (7)$$

where C represents  $^{12}\text{C}$  and  $C^*$  represents  $^{13}\text{C}$ , the first term on the right side of eqs. (6 and 7) represents vertical diffusion of  $^{12}\text{C}$  and  $^{13}\text{C}$  (where D is the diffusion constant), the second term on the right side represents vertical advection of  $^{12}\text{C}$  and  $^{13}\text{C}$  (and v the advection constant),  $kC$  and  $kC^*$  represent first-order turnover (heterotrophic respiration) of soil C, and  $f_d$  represents the rate of C inputs from plant roots. The isotopic fractionation constant is denoted by  $\alpha$  and the  $^{13}\text{C}/^{12}\text{C}$  ratio in root inputs is denoted  $R_1$ .

After specifying boundary conditions (including plant-litter inputs of C at the soil surface) and introducing other assumptions including the approximation that root-litter inputs are zero and that the system is in steady state, the authors found an analytical solution. The solution amounted to an equation describing changes in R, the ratio of  $^{13}\text{C}/^{12}\text{C}$ , with depth (z):

$$R(z) = \frac{R_p}{\sqrt{\alpha}} e^{(\sqrt{k/D})(z\sqrt{\alpha}-1)} \quad (8)$$

where  $R_p$  is the ratio of  $^{13}\text{C}/^{12}\text{C}$  in plant litter inputs at the surface. This solution was used to calculate modeled changes in  $\delta^{13}\text{C}$  with depth. The  $\delta$  values were then compared directly against field observations, both to assess the model and to provide a theoretical explanation of the field observations in terms of the conceptual processes represented by the equations: diffusion, advection, and turnover. (The authors pointed out that “diffusion” is intended in an abstract sense and probably includes biotically-driven mixing across soil depths.) This highlights a key shortcoming in the use of differential equation models: It is the nature of such analysis to introduce concepts such as diffusion and steady-state into the model and the analysis because analytical solutions can then be obtained.

An exception to the typical use of differential equations in isotope modeling is the NESIS model (Non-Equilibrium Stable Isotope Simulator) developed by Rastetter et al. (2005). A goal in developing NESIS was to help “overcome the problems associated with the (often implicit) assumption of steady-state used in many isotope studies” (Rastetter et al. 2005). One of the most promising aspects of the NESIS model is that it can essentially add isotopically-explicit calculations for any element or elements to an existing nonlinear, dynamic model of ecosystem biogeochemistry (referred to as the “parent model”). NESIS runs independently of the parent model. For inputs, NESIS requires values of all of the pools and fluxes of an element at the start and end of each timestep in the parent model, isotopic ratios in each pool at the start of the simulation, isotopic fractionation constants associated with each flux, and any external inputs to any pool over time together with their isotopic signatures (which can include enriched tracers). NESIS then uses a linear, donor-controlled set of differential equations to describe isotopic ratios in each pool of the parent model in each timestep and to calculate separate fluxes of the heavy and light isotope associated with each flux in the parent model. The set of simultaneous equations describing heavy and light isotope fluxes are solved, in each timestep, through a numerical integration technique. Nonlinear, non-steady-state dynamics can be accommodated because NESIS self-corrects by re-reading all pool sizes and flux values from the parent model in each timestep and re-estimates the parameters for the linear, donor-controlled model. This is a promising direction in isotope modeling. Although most biogeochemical models do not print out enough information in each timestep for NESIS, they could be altered to do so much more easily than they could be rewritten to include isotopically-explicit internal equations.

### **Algorithmic process models**

In causative-dynamic models of ecosystem biogeochemistry, equations or algorithms that represent individual ecological or biogeochemical processes such as photosynthesis, nitrification, or evapotranspiration are linked together in chains of cause and effect. Within this category, models vary significantly in the degree of empiricism or mechanism in their formulations of individual processes as well as in model complexity. By including ecosystem-level feedbacks in element cycles and other nonlinear processes these models can be used to investigate interactions across levels of organization. Investigators are able to simulate interactions among individual causal-mechanistic processes that give rise to emergent properties at the ecosystem level or at the level of key subsystems. This approach typically involves construction of a mechanistic set of cause-and-effect relationships first written as algebraic or differential equations. These are translated into difference equations with a discrete time step and translated further into a set of computer instructions (an algorithm) to simulate the system on a discrete time interval (Currie et al. 1999). This produces a dynamic, nonlinear evolution of state

variables. Because analytical solutions are not sought, these models can include element (including tracer) recycling and non-steady-state dynamics, requiring fewer such assumptions relative to analytical models (Fig. 5). The use of algorithmic computer models to simulate ecosystem biogeochemistry in this manner has been popular for decades (e.g. Pastor and Post 1986, Parton et al. 1988). The use of such an approach to simulate redistributions of stable isotopic tracers for ecosystem biogeochemistry has grown over the last decade (Tietema and Wessel 1992, van Dam and van Breemen 1995, Tietema and van Dam 1996, Koopmans and van Dam 1998, Currie et al. 1999, Hobbie et al. 1999, Currie et al. 2004). An important reason to consider using such a model is that well-known interactions between element cycles can be included as well as the effects of well-understood forcing functions such as temperature and moisture. An isotopically-explicit formulation of a model, when combined with isotope measurements, makes it possible to test model mechanisms more fully and thus increase confidence in the model for non-isotope applications.

As an isotopically-explicit model runs, it requires four pieces of information for each elemental pool (e.g. for each pool of N): the total elemental pool size (e.g.  $^{14}\text{N} + ^{15}\text{N}$  in  $\text{g N/m}^2$ ), the mass of  $^{14}\text{N}$  (in  $\text{g }^{14}\text{N/m}^2$ ), the mass of  $^{15}\text{N}$  (in  $\text{g }^{15}\text{N/m}^2$ ), and the ratio of  $^{14}\text{N}/^{15}\text{N}$  in the pool. For a number of reasons, including minimizing computer round-off errors, it is best to store and continually update only two pieces of information and calculate the other information as needed in the model. The model could store the  $^{14}\text{N}$  mass and  $^{15}\text{N}$  mass in each pool and calculate the total N and the  $^{15}\text{N}/^{14}\text{N}$  ratio when needed; alternatively, the model could store the total N and the isotopic ratio in each pool and calculate the  $^{14}\text{N}$  mass and  $^{15}\text{N}$  mass when needed. In the TRACE model (Tracer Redistributions Among Compartments in Ecosystems, Currie et al. 1999), written in the MicroSoft VisualBasic language,  $^{14}\text{N}$  and  $^{15}\text{N}$  masses for each pool are stored in a 1-dimensional vector variable. For example,  $^{14}\text{N}$  and  $^{15}\text{N}$  masses (in  $\text{g/m}^2$ ) in a pool of N named *NPoolA* would be stored as *NpoolA(1)* and *NpoolA(2)*, respectively, where the indices ( $i = 1$ ) and ( $i = 2$ ) allow two different values to be stored in the vector variable *NpoolA(i)*. The proportions of  $^{14}\text{N}$  and  $^{15}\text{N}$  in this pool (here denoted by the variable names *f14NpoolA* and *f15NpoolA*, respectively), and the total N in the pool (*TotNpoolA*) would be recalculated whenever needed, using the following code:

$$\begin{aligned} \text{TotNpoolA} &= \text{NpoolA}(1) + \text{NpoolA}(2) \\ \text{f14NpoolA} &= \text{NpoolA}(1) / (\text{NpoolA}(1) + \text{NpoolA}(2)) \\ \text{f15NpoolA} &= \text{NpoolA}(2) / (\text{NpoolA}(1) + \text{NpoolA}(2)) \end{aligned} \quad (9)$$

Note that these are ‘assignment’ statements, in which the quantity on the right side of the equation is calculated and then stored in the variable named on the left side of the equation. A gross flux of total N between *NPoolA* and *NPoolB*, named *NfluxAB* and defined as positive in the direction from A to B (thus negative if from B to A), would be carried out as follows, debiting the source or donor pool and crediting the sink, or target pool in an isotopically-explicit manner:

$$\begin{aligned} \text{If } \text{NfluxAB} > 0 \text{ then} & & (10) \\ \quad \text{NpoolB}(1) &= \text{NpoolB}(1) + \text{NfluxAB} * \text{f14NpoolA} \\ \quad \text{NpoolB}(2) &= \text{NpoolB}(2) + \text{NfluxAB} * \text{f15NpoolA} \\ \quad \text{NpoolA}(1) &= \text{NpoolA}(1) - \text{NfluxAB} * \text{f14NpoolA} \\ \quad \text{NpoolA}(2) &= \text{NpoolA}(2) - \text{NfluxAB} * \text{f15NpoolA} \\ \text{Else} & \\ \quad \text{NpoolA}(1) &= \text{NpoolA}(1) + \text{NfluxAB} * \text{f14NpoolB} \\ \quad \text{NpoolA}(2) &= \text{NpoolA}(2) + \text{NfluxAB} * \text{f15NpoolB} \\ \quad \text{NpoolB}(1) &= \text{NpoolB}(1) - \text{NfluxAB} * \text{f14NpoolB} \\ \quad \text{NpoolB}(2) &= \text{NpoolB}(2) - \text{NfluxAB} * \text{f15NpoolB} \\ \text{End if} & \end{aligned}$$

Note that according to the principle of pool dilution, the isotopic ratio of the gross flux between *NPoolA* and *NPoolB* is equal to the isotopic ratio of the source pool, which varies according to the direction of the gross flux. This coding approach lends itself to the process of augmenting an existing

model to add isotopically-explicit calculations. For example, if an existing model had the pool  $N_{poolA}$  as a variable, this could simply be replaced by a vector variable of the same name, initialized with a background isotopic ratio, and all model algorithms amended to include the principles of pool dilution and mass balance in each isotope. For a simple model this could be straightforward, but for a complex model this substantially increases the amount of code and the level of model complexity. Note that in a non-isotope model of N cycling, the 11 lines of the code in eq. (10) could be accomplished with two, much simpler, lines of code. Process models implement the principle of pool dilution by recalculating the proportions such as  $^{14}\text{N} / (^{14}\text{N} + ^{15}\text{N})$  and  $^{15}\text{N} / (^{14}\text{N} + ^{15}\text{N})$  in each model pool after each time step (van Dam and van Breemen 1995) or, alternatively, in a source pool prior to any gross flux of N and the target pool after any gross flux of N (Currie et al. 1999). Isotopically-explicit models provide the ability to simulate gross (as well as net) elemental fluxes; at the same time, they require the simulation of gross elemental fluxes if they are to incorporate the principle of pool dilution.

Isotopic fractionation has been either included or excluded from particular algorithmic process models. In using the NICCCE model (Nitrogen Isotopes and Carbon Cycling in Coniferous Ecosystems; van Dam and van Breemen 1995), which included both isotopic fractionation and enriched-tracer redistributions for N, Koopmans and van Dam (1998) concluded that fractionation was insignificant to model simulations when enriched tracers were well above natural-abundance levels. Currie et al. (1999) used this assumption in the development of the TRACE model, which omitted fractionation to simplify the modeling of the redistributions of highly enriched tracers.

When fractionation is included, the mathematics is often simplified by approximating the behavior of the more common isotope of an element (i.e.  $^{12}\text{C}$  or  $^{14}\text{N}$ ) as equal to that parameterized for the total element (i.e. C or N). For example, in the NICCCE model, Koopmans and van Dam (1998) described generalized flux calculations for  $^{14}\text{N}$  and  $^{15}\text{N}$  (denoted  $J_{14N}$  and  $J_{15N}$  respectively) as:

$$J_{14N} = K(^{14}\text{N} + ^{15}\text{N})(1 - R) \quad (11)$$

$$J_{15N} = K(^{14}\text{N} + ^{15}\text{N})R(1 - \alpha) \quad (12)$$

where  $R$  is the ratio ( $^{15}\text{N} / (^{14}\text{N} + ^{15}\text{N})$ ),  $\alpha$  is the fractionation constant, and  $K$  is a reaction rate constant that is multiplied by the mass of N in the donor pool.  $K$  is parameterized or defined for whole-element (i.e. total N) fluxes. Substituting the value for  $R$  in eqs. (11 and 12) and canceling terms, they simplify to:

$$J_{14N} = K^{14}\text{N} \quad (13)$$

$$J_{15N} = K^{15}\text{N}(1 - \alpha) \quad (14)$$

This illustrates that the reaction-rate constant  $K$  is applied to  $^{14}\text{N}$  mass alone, but the ratio of fluxes  $J_{14N}$  and  $J_{15N}$  is correctly preserved. This approximation introduces a small error in whole-element cycling rates (Amundson and Baisden 2000), but this error is well beyond the precision with which reaction-rate constants are typically specified.

The decision whether or not to include fractionation impacts model uncertainty either way. Incorporating fractionation processes in a complex ecosystem model adds an additional set of parameters, i.e. fractionation rates, each with an additional uncertainty (Hobbie et al. 1999, van Dam and van Breemen 1995). Another consideration is the metric that will be used in model-data comparisons. Depending on the level of tracer enrichment, it may not be advisable to omit fractionation processes in the model if values of  $\delta^{15}\text{N}$  will be compared directly against observations, whereas it may be appropriate if percent recoveries of  $^{15}\text{N}$  tracers are compared. Omitting fractionation rates could impact model results more significantly over longer time periods or as tracers become more dilute in ecosystem pools. Finally, an additional and related source of uncertainty is the problem of kinetic fractionation. Isotope models typically employ thermodynamic fractionation constants, i.e. differences in reaction rates when both

isotopes are plentiful in the source or donor pool. In nature, an important effect is the kinetic change in fractionation, in which an isotopic difference in reaction rates decreases as the size of the source pool decreases (Schimmel 1993). Representing such effects has not received much attention in the modeling of stable isotopes.

Complex model code should be tested for errors prior to use in planning or interpreting field experiments. Subtle errors can arise in logic, in the translation of equations to algorithms, and in code writing. Computer round-off errors can also arise and propagate because some computations are repeated numerous times. Error-checking can involve operational tests performed by the user and can also involve model enhancements to include automated error-checking code. A common operational test is to run a model with all isotope calculations active, but all pools initialized to the isotopic ratio of the standard, and all fractionation constants set to zero (Hobbie et al. 1999, Currie et al. 2004). Values of  $\delta$  should then remain zero throughout the simulation. In this test, computer round-off errors can lead to a nonzero value in the 2<sup>nd</sup> decimal place of  $\delta$ , e.g.  $\delta^{15}\text{N} = 0.02 \text{ ‰}$  when it should be 0.00 ‰. This is insignificant in model-data comparisons because it is an order of magnitude below the precision with which  $\delta^{15}\text{N}$  is typically specified.

An example of a semi-automated test is an ecosystem mass-balance test for each element. In a simple approach to test mass balance for N, initial total ecosystem N is calculated by summing all N pools at the start of the model run. A cumulative ecosystem-level input-output balance of N is saved as a bookkeeping variable, initialized to zero at the start of the model run and incremented any time there is an N influx or outflux (positive values for influxes). Total ecosystem N can be calculated at the end of each timestep by summing all N pools in the model; when the cumulative input-output flux of N to that timestep is subtracted, the difference should equal the initial value of total ecosystem N. This test can be incorporated into model code easily for each element and printed and checked by the user prior to or during each model run (Currie et al. 2004). Another simple semi-automated test is to check that values of percent recovery of tracers sum to 100% in the model, when corrected for ecosystem losses of the tracer. Introducing model code for fully automated tests is more involved but potentially important. In the code of the TRACE model, for example, automated tests are performed to raise an error flag if any pool of  $^{14}\text{N}$  or  $^{15}\text{N}$  mass becomes less than zero at any point in time (with the exception of computer round-off errors). Under normal operation this never occurs, but in Monte-Carlo simulations in which numerous model parameters are varied stochastically and independently (Currie and Nadelhoffer 1999), infrequently a random combination of parameter values causes a slight violation of mass balance to develop, which is then flagged by the error-checking code and the model run is automatically discarded.

### **Sources and analysis of uncertainty in interpreting field study results**

While the sensitivity of stable-isotope ratios to gross elemental fluxes and to fine-scale processes provides an additional dimension of observation, it can also create difficulties in interpreting natural-abundance patterns or tracer movements. The dynamics of an isotopic ratio in an ecosystem pool provides only a signature of the net effects of multiple processes that may be interwoven. This can hinder interpretation of elemental movements and transformations where multiple pathways are possible, where pool sizes are inadequately known, or where detailed mechanisms are poorly understood. While this limitation is true of both empirical and modeling studies (Nadelhoffer et al. 1994), it may seem more vivid in a modeling analysis. In this section I consider uncertainty and limitations inherent in stable-isotope modeling as well as some approaches, such as inverse modeling or iterative model-data comparisons, that have been used to overcome these limitations.

In complex isotopically-explicit models, uncertainties arise from a somewhat different set of sources and approximations than in simple models. In model-data comparisons using either type of model, uncertainties are present in standard errors of measurement (SEM) of background isotopic ratios, in SEM of observed isotopic ratios through time, in SEM of elemental pool sizes, and in the degree of success in labeling a particular pool with an enriched tracer (if used). Algebraic and some differential-

equation models require a greater number of assumptions such as steady-state, no tracer recycling, and linear interactions or near-equilibrium conditions. These assumptions contribute to uncertainty in model-data comparisons (Tietema and Wessel 1992). Many of these assumptions can be dropped in algorithmic simulation models, but at the same time, other types of assumptions and approximations increase (Fig. 5). These include assumptions about conceptual model structure (which increase necessarily as the number of pools and fluxes in the model increase) and assumptions made in specifying values for parameters (which increase in number rapidly as the process-based complexity of the model increases). An additional source of uncertainty in algorithmic models that is not present in analytical models (where the latter employ steady-state) lies in temporal scaling of dynamic processes. If the dynamics of a gross flux in nature is much more rapid than the model timestep (e.g. minutes or hours for microbially-mediated processes, versus a monthly timestep in a model), the model must endeavor to make an empirical approximation of the isotopically-explicit effects of the gross flux on a monthly basis – which may be a coarse approximation.

Another key source of model uncertainty lies in sensitivity to parameters that are difficult to quantify in the field or to historical initial conditions that are difficult to estimate. For example, C and N cycling processes in the TRACE model are sensitive to turnover rates of humified matter in the organic and mineral soil horizons (Currie et al. 1999). Unfortunately, turnover rates of humified matter *in situ* are very difficult to quantify meaningfully in a process-based field study and are typically estimated in a coarse manner from other data (e.g. Currie and Aber 1997). Specifying historical initial conditions with confidence is equally problematic (Yanai et al. 2003). Even at locations where site management and disturbance histories are well known, the biogeochemical and ecological history is typically not known precisely enough to initialize past pool sizes of C and N, or to set the historical rates of NH<sub>4</sub> and NO<sub>3</sub> deposition, or to test the modeled vegetation history against historical rates of key ecosystem processes such as photosynthesis or nutrient uptake (Currie et al. 2004). Investigators infer or extrapolate such historical pool sizes and fluxes from present-day observations and from other historical data; in an environment that has seen dramatic change in the past century, such inferences are made with limited confidence.

One approach to confront such uncertainty is to adopt precise best-estimates of initial or past conditions and conduct several comparative model runs in a formal or informal analysis of model uncertainty. For a complex algorithmic model, Monte-Carlo techniques can be used. Van Dam and van Breemen (1995) assessed the range of model predictions that resulted when 52 parameters were varied stochastically by  $\pm 20\%$ ; similarly, Currie and Nadelhoffer (1999) assessed model uncertainty when 63 parameters varied stochastically in normal distributions with standard deviations of 10%. This formal analysis, while important because it includes nonlinearities in the model, addresses only one aspect of model uncertainty, i.e. that arising from confidence limits on parameters. An overall assessment of uncertainty in model-data comparisons would need to combine the results of a parameter-based analysis with uncertainties in observations such as pool sizes, together with an analysis of uncertainties in model structure (Rastetter 2003) and an analysis of model results under ‘bracketed’ historical conditions (e.g. Goodale et al. 2003). Even with such a thorough analysis of uncertainties, a modeling analysis would contain additional assumptions and approximations, for example the assumption that elemental pools are homogeneous and well-mixed and the assumption that kinetic isotopic fractionation (Schimel 1993) can be neglected.

### **Over-determinacy**

When a model has a large number of parameters that are difficult to specify with confidence, relative to the number of model predictions that can be compared against observations, the model might be referred to as being mathematically “over-determined”. In this situation, agreements between model results and field observations could be obtained with numerous different parameter sets that one might use. This is the case not only for isotopically-explicit models but for virtually all complex models of ecosystem biogeochemistry. Parsimony in model construction can help to combat this problem (Hobbie et al. 1999).

The tradeoffs include omission of interactions with other element cycles or physical environmental variables (e.g. temperature, moisture), or simplification of pools and fluxes in the conceptual model at the expense of realism (e.g. combining microbial and detrital material into a single pool; Currie et al. 1999). Over-determinacy can be an ever-growing problem because as an already complex model is enhanced and applied to new situations there is a tendency to include additional processes and mechanisms (Bankes 1993).

Given a model structure and number of parameters, at least three techniques have been used to address over-determinacy. Each of these techniques has drawbacks. First, a multivariate statistical technique can be used to simultaneously calibrate numerous parameters while minimizing a statistic, such as the sum of squared deviations, in a direct model-data comparison (Rastetter et al. 1992). The second technique involves a Monte-Carlo randomization of parameter values, with rejection of a large number of model runs in which certain key results are outside of the confidence range of field-observed values (van Dam and van Breemen 1995). A third technique makes use of stepwise calibration of a small number of sensitive parameters that are difficult to quantify empirically, while comparing a larger number of key pools and fluxes against field-measured values (Currie et al. 2004). Drawbacks with these approaches are that a test statistic for optimization of model-data agreement or thresholds of confidence used for parameter-set rejection may seem arbitrary and the resulting parameter set may not be unique. Additionally, over-use of calibration techniques creates a circularity if all of the available data to test a model are used to calibrate the model.

Incorporating isotopes into a complex model can either exacerbate the problem of over-determinacy or help to mitigate the problem. The additional dimension of observational data (an isotopic ratio) should help to combat the problem, but the number of additional model parameters may grow faster than the number of additional observations. Keeping the number of conceptual pools and fluxes no larger than necessary, and close to the operationally-defined observables should help, but a tradeoff is that an operational field or laboratory method may measure conceptually inconvenient quantities. One approach taken by two teams of investigators using complex isotopically-explicit ecosystem models (van Dam and van Breemen 1995; Currie et al. 2004) has been to parameterize and calibrate overall pool sizes and fluxes of N-cycling first, without reference to N-isotope observations, and then to predict dynamics in isotopic ratios. This has the benefit of ensuring that the magnitude of nutrient cycling pools and fluxes are realistic for a particular field study, while allowing the modeled stable-isotope redistributions, which are sensitive to model mechanisms, to be falsifiable predictions (Currie et al. 2004).

## **Summary and conclusion**

The greatest present strength of isotopically-explicit biogeochemical models lies in aiding the interpretation of field and laboratory observations – this reflects the main purpose for which such models have been developed. The use of mathematical models to elucidate the ramifications of mass balance or pool dilution in simple one-box or two-box models, or slightly more complex models that disallow tracer recycling, is well standardized and widely used. Complex ecosystem models that include stable isotopes, both at enriched-tracer and natural-abundance levels, have been applied to interpret observational data from large-scale field studies, although these models are still in relatively early stages of advance. Some areas of model development and application could still be considered to be in experimental or exploratory stages. These include, for example, the combination of isotopic fractionation and enriched-tracer redistributions in the same model, or the combination of multiple stable isotopes of different elements in the same model.

A legitimate question to ask is the following: Why should one add more processes or more detail to a model if this exacerbates problems that complex models already face regarding parameterization? Oreskes (2003) has argued convincingly that when more processes are included in a model to increase its realism, our uncertainty increases regarding whether our tests of the model are adequate or meaningful. The inclusion of isotopes in a model may provide a counter-example because this provides an additional

set of state variables to test against observational data – it provides a clear constraint on elemental flows in ecological systems of all kinds. Because the new state variables (ratios of stable isotopes) are highly sensitive to mechanistic processes, when used in combination with existing state variables their inclusion could increase our confidence in the model relative to an isotope-free formulation. A model with isotope ratios, even though it contains additional complexity and parameters, has the potential to be more fully testable. For example, we might have greater confidence in a model of C pools and fluxes if it simulated not only testable  $^{13}\text{C}/^{12}\text{C}$  ratios in its pools of C, but also C/N interactions and testable  $^{15}\text{N}/^{14}\text{N}$  ratios in its pools of N. Extending this idea, we might have increased confidence in a C model if it also included energy fluxes, H and O fluxes and isotopes in water vapor and biomass, and so on. Reiners advanced this idea as the principle of using ‘complementary’ models in the study of ecosystems (Reiners 1986). In the practical aspects of coding and parameterizing models, there are tradeoffs between the Reiners’ principle of complementarity and Oreskes’ argument from epistemology. It may not be worthwhile to add new dimensions to a model if, in each case, so many new parameters would need to be added that the problems of over-determinacy, sensitivity to unknown quantities, and unclear interactions among model processes were exacerbated (Bankes 1993).

Computational, conceptual, and philosophical advances are all needed in stable-isotope modeling. Continued advance is needed in the development of mathematics and algorithms. For example, concisely-formulated computer algorithms are needed that will be able to simulate isotopic fractionation and enriched tracer redistributions in sets of interacting processes, potentially with isotopes of multiple elements at once. The complications in encoding algorithms grow rapidly with each new set of processes that are added to an already complicated model. Unfortunately, few new investigators in ecology and biogeochemistry are receiving training in the computational tools and techniques needed to make these advances. While the ‘democratization’ of modeling, training more ecologists to understand and to use models (Cottingham et al. 2003), is highly desirable, it is likely that the development of concise algorithms for advanced stable-isotope modeling will be pursued by investigators who specialize to some degree in informatics and computation.

The second need for advance is conceptual. A continued merging of conceptual models and empirical (field and laboratory) methods is needed so that inconsistencies can be removed. Improvement is needed on both ends. Conceptual models are needed that more accurately represent operationally-defined observations that are made in field studies. At the same time, operationally-defined observations are required that come closer to our theoretical ideas about the causative mechanisms that we aim to incorporate into causative-mechanistic models.

Third, progress is needed on methodological questions related to modeling approaches. How will we overcome the problem of over-determinacy in complex models while maximizing our ability to gain increased insight and understanding into complex natural systems? Ecologists sometimes adopt the stance that the only informative modeling results are those that can be shown to agree significantly with observations in a traditional or “frequentist” (Reckhow 2003) statistical approach (e.g. Gardner and Urban 2003). While such a criterion is important to consider, it is not a necessary condition for complex modeling analyses to be useful. Field observations themselves are subject to uncertainty. Observational data at times reflect idiosyncrasies of field measurement methods rather than a clear correspondence with a conceptual pools or fluxes (Yanai et al. 2003). Observational data may also reflect idiosyncrasies of a particular year in which measurements were made, not necessarily representative of longer time periods (McClain et al. 2003), with no means of assessing that fact from the limited data themselves. When observations of disparate pools and fluxes, collected by various investigators for various purposes, are assembled in an attempt to synthesize a view of elemental cycling in a particular system, the resulting dataset may lack internal self-consistency (for example, field-observed percent recoveries of  $^{15}\text{N}$  tracers typically do not sum to 100%). If we required synthetic process models to meet both the criteria that (a) all modeled pool and flux values must match field data within a traditional statistical uncertainty and (b) the model must be self-consistent mathematically, these could be mutually exclusive requirements.

Clearly, methodological progress is needed in the manner in which we use models to gain increased insight into natural systems. The problem I refer to here as over-determinacy is a serious hurdle that will require creative solutions to overcome. The heuristic value of inverse modeling or exploratory modeling are key areas for future research (Banks 1993, Luo et al. 2001, Currie et al. 2004). Future research should also explore the use of alternative statistical underpinnings, such as Bayesian inference, that allow the incorporation of multiple modes of evidence including indirect evidence supplied by complex causative models (Reckhow 2003). Progress is needed in developing computational approaches, in improving consistency between conceptual models and observational techniques, and in developing creative methodological approaches to combine evidence from field and modeling investigations. Since the field of isotope modeling in ecosystem biogeochemistry is young, much progress is possible in each of these areas.

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## Figure captions

Fig. 1. A conceptual model is used both to guide observations and to guide the construction of a mathematical model, which is then used to interpret observations.

Fig. 2. Results of a simple model illustration: Panel A shows pools and fluxes of N in  $\text{g m}^{-2}$  and  $\text{g m}^{-2} \text{ timestep}^{-1}$ , respectively, for steady-state N fluxes and pool sizes shown. Panel B shows the value of  $\delta^{15}\text{N}$  in pool  $P_a$  over time, given a 1000‰  $^{15}\text{N}$  tracer introduced into pool  $P_a$  at time zero. Here,  $\delta^{15}\text{N}$  begins at zero and remains at zero in pool  $P_b$ . (Contrast with Fig. 3.) Note logarithmic scale on the  $\delta^{15}\text{N}$  axis.

Fig. 3. Results of a simple model illustration: Panel A shows pools and fluxes of N in  $\text{g m}^{-2}$  and  $\text{g m}^{-2} \text{ timestep}^{-1}$ , respectively, for steady-state N fluxes and pool sizes shown. Panel B shows the values of  $\delta^{15}\text{N}$  in pool  $P_a$  and  $P_b$  over time, given a 1000‰  $^{15}\text{N}$  tracer introduced into pool  $P_a$  at time zero. Here,  $\delta^{15}\text{N}$  begins at zero in pool  $P_b$  and rises to 40‰ within 5 timesteps. Note that the net flux of N from  $P_b$  to  $P_a$  is identical to that in Fig. 1, while the gross flux from  $P_b$  to  $P_a$  is  $10\times$  greater. Axes and scales are as in Fig. 2.

Fig. 4. A two end-member mixing-model conceptualization in which  $R_M$ , the measured isotopic ratio in a pool, is conceived as a mixture of the two end-member isotopic ratios  $R_A$  and  $R_B$ .

Fig. 5. Relative relationships among numbers of assumptions of various types that are needed by models incorporating principles of isotopic “pool dilution” and mass balance.

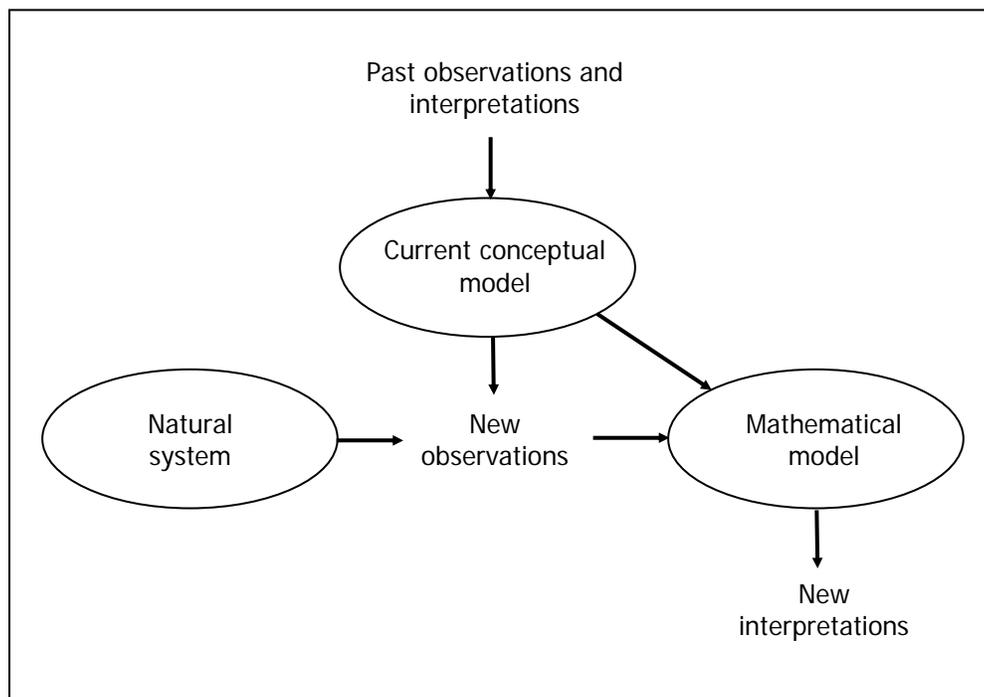


Figure 1

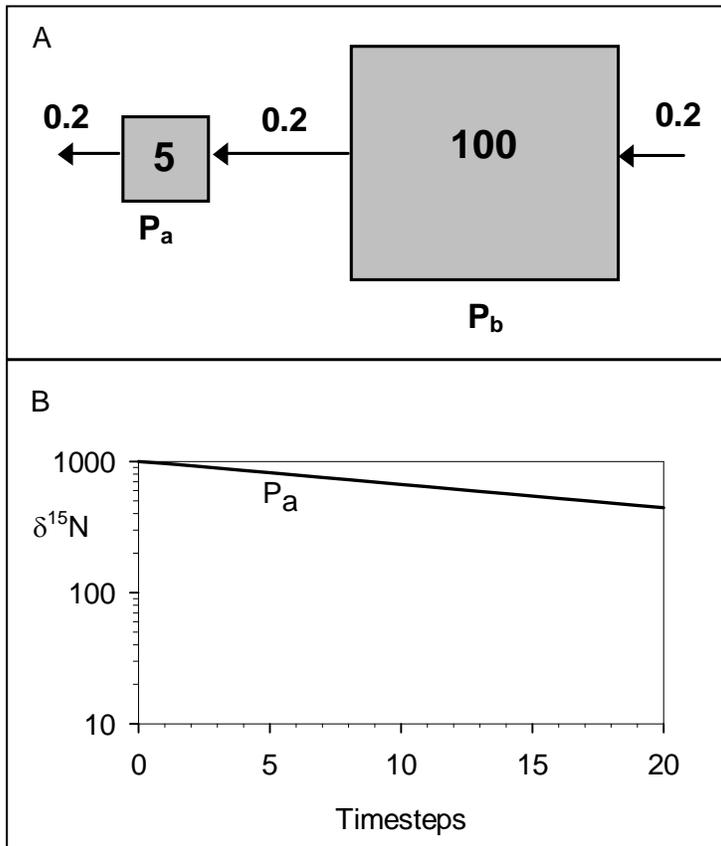


Figure 2

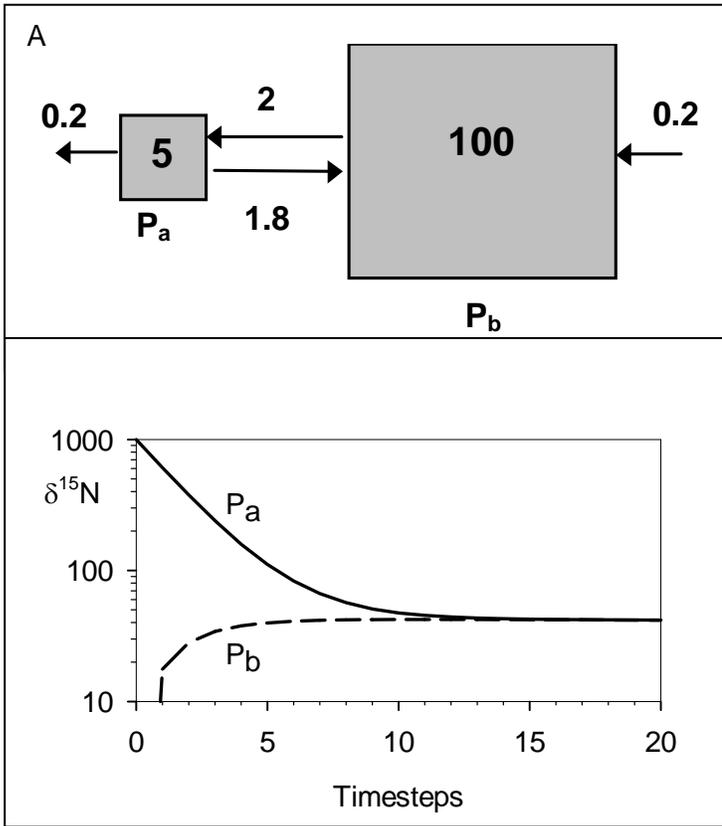


Figure 3

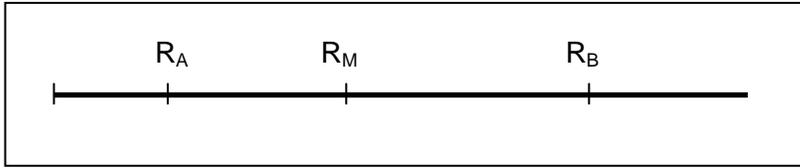


Figure 4

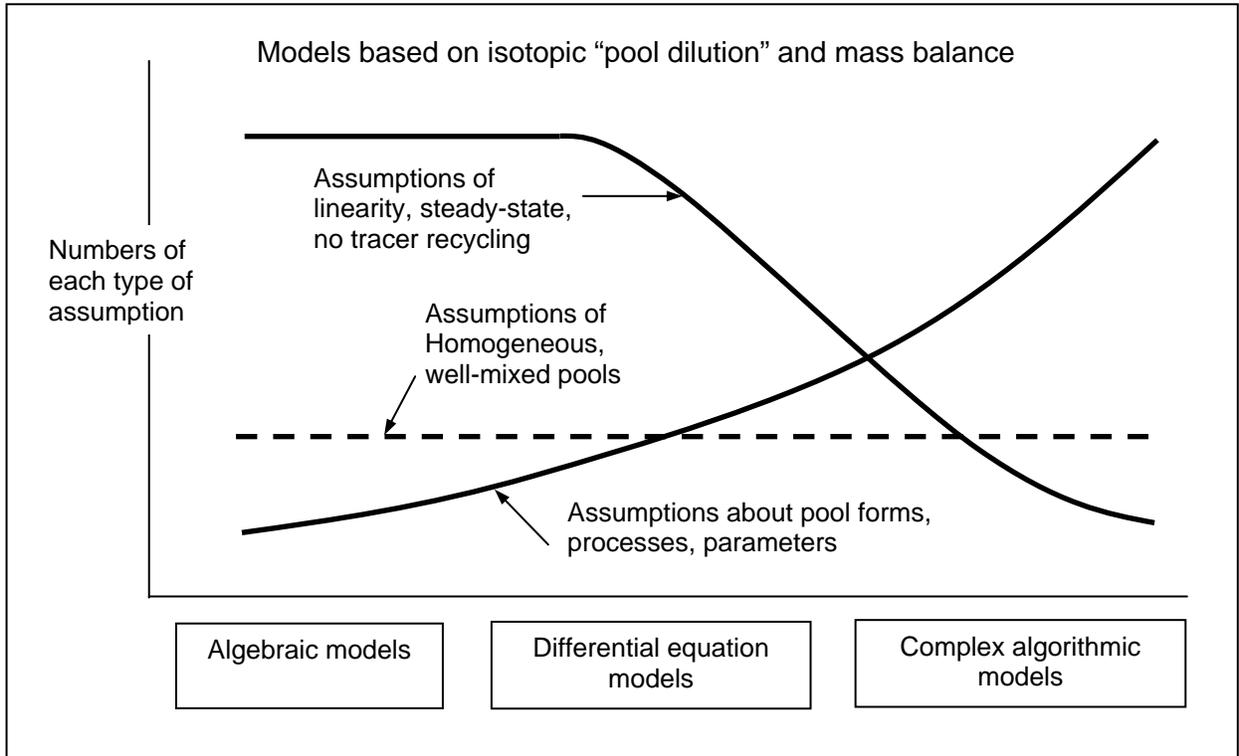


Figure 5